The Archives of Internal Medicine

Vol. XIII FEBRUARY, 1914 No. 2

STUDIES ON THE CIRCULATION IN MAN

XI. THE BLOOD-FLOW IN THE HANDS AND FEET IN CERTAIN DISEASED CONDITIONS OF THE VESSELS OR OF THEIR VENOUS MECHANISM *

G. N. STEWART, M.D.
CLEVELAND

I. ARTERIOSCLEROSIS

It need scarcely be pointed out that several of the patients in the previous paper suffered from arteriosclerosis, in addition to valvular lesions of the heart or myocardial changes. In this section a few cases are brought together in which arteriosclerosis was pronounced and other lesions if present did not dominate the clinical picture. As a general result of the observations, it may be stated that in marked arteriosclerosis the flow in the hands is always smaller and the vasomotor reflexes weaker than in normal persons. Vasodilatation is easier to obtain by the application of warmth to the contralateral hand than vasoconstriction by the application of cold.

One of the purest cases of marked arteriosclerosis is that of Meyer G., a man aged 39, without detectable cardiac involvement. The flow was only 2.88 grams for the right hand and 3.46 grams for the left hand per 100 c.c. of hand per minute, with room temperature 22 C. An increase to 3.96 grams in the right was occasioned by immersion of the left hand in warm water, and the flow only fell to 3.52 grams when the left hand was subsequently put into cold water. It must not be forgotten, however, in connection with the small flow in this case that the man was a peddler accustomed to expose his hands in the open air in winter. Also he was a dispensary patient, and came from his work on a day in February.[1]

Meyer G., Hebrew peddler, aged 39, height 5 feet 6½ inches, was admitted at the dispensary Sept. 3, 1910, with marked arteriosclerosis. There was great thickening of all accessible arteries. The heart examination was negative in result. He complains of having had pain in the stomach during the past year. A test-meal showed free hydrochloric acid. No masses are palpable in the abdomen. He attributes his condition to strain in pushing heavy loads on his hand-cart. The hand flow was measured Feb. 3, 1911. He still complains of pain

* From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.

1. See Paper IX of this Series, THE ARCHIVES INT. MED., 1913, xii, 678.

in the epigastrium. On emptying capillaries on the back of the hand by pressure the color returns very slowly. Pulse, 72. Mouth temperature, 36.7 C. Systolic pressure in the right brachial, 110. Hands put into bath at 2:49 p. m.; into calorimeters at 2:59½.[2] Volume of right hand in calorimeter, 525 c.c.; of left hand, 525 c.c. He is right-handed. His hands are large. (Table 1.)

Another outside worker (John O'R.), a street laborer aged 60, with a diagnosis of arteriosclerosis and myocarditis, had a flow of 5.49 grams per 100 c.c. per minute in the right hand and 5.44 grams in the left hand with room temperature 23 C. The contralateral vasoconstriction to cold

TABLE 1.—Calorimetric Measurements in Case of Meyer G.

Time	Right	Left	Notes	Time	Right	Notes
2:58½	29.74	29.81		3:28	29.98	
3:01	29.69	29.75		3:29	30.00	
3:02	29.70	29.74	Room temp. 22.2 C.	3:30	30.02	
3:03	29.70	29.74		3:31	30.07	At 3:31 left hand put in water at 7.2 C.
3:04	29.70	29.74				
3:05	29.70	29.75		3:32	30.09	
3:06	29.70	29.75		3:33	30.11	
3:07	29.69	29 76		3:34	30.13	
3:08	29.69	29.75		3:35	30.16	
3:09	29.69	29.75		3:36	30.18	
3:10	29.69	29.78	Room temp. 22.3 C.	3:37	30.20	Room temp. 21.9 C.
3:11	29.71	29.80		3:38	30.21	
3:12	29.72	29.81		3:39	30.22	
3:13	29.73	29.82		3:40	30.23	
3:14	29.75	29.85		3:41	30.25	
3:15	29.77	29 89		3:42	30.27	At 3:42 left hand dried and wrapped in warm cloth.
3:16	29.78	29.90				
3:17½	29.80	29.93		3:43	30.29	
3:19	29.82	29.97		3:44	30.31	
3:20	29.83	30.00		3:45	30.33	
3:21	29.86	30.01	Room temp. 21.7 C.	3:46	30.36	Room temp. 22 C.
3:22	29.88	30.03		3:47	30.39	
3:23	.....	30.08	At 3:23 left hand put in water at 43 C.	3:48	30.42	
				3:49	30.45	Room temp. 22.2 C.
3:24	29.90	.....		3:50	30.48	At 3:50 hand out of calor.
3:25	29.92	.....		3:51	.....	Temp. of L. is now 29.75 C.
3:26	29.94	.....	Room temp. 21.6 C.	4:16	30.13	
3:27	29 96	.....		4:17	.....	Temp. of L. is now 29.48.

was slight, the flow in the right hand diminishing from 5.49 grams to 4.78 grams, but warmth gave a fair vasodilatation after the usual preliminary vasoconstriction. The protocol has already been published.[3]

Charles S., a man aged 69, with sclerosed vessels, high blood-pressure and enlarged cardiac dulness, but no murmurs, had a flow in the right hand of only 5.25 grams per 100 c.c. per minute and in the left a flow of 5.01 grams, with the high room temperature of 28.3 C. The response

2. In all the observations unless otherwise stated the quantity of water in each hand calorimeter was 3,015 c.c.

3. Cleveland Med. Jour., 1910, x, 385.

of the vessels to reflex impulses from the contralateral hand was very poor, cold producing a small diminution in the flow, warmth no response whatever beyond the initial flow.

Charles S., a hammer man, aged 69, was admitted to Lakeside Hospital, June 26, 1911. He had gonorrhea forty-five years ago; never any chancre. He has been a heavy coffee and tea-drinker except in the last four or five years. He seldom drank whisky or beer, and smokes little. Six years ago following an attack of pneumonia he ate a heavy meal and immediately felt short of breath, dizzy and faint. He has had dizzy spells ever since. The attacks of dyspnea are worse at night and come on suddenly. Lately there has been swelling of the feet and ankles. There is Cheyne-Stokes respiration with periods of complete apnea lasting forty seconds. The anteroposterior diameter of the thorax is increased. There is difficulty in expelling air from the lungs. The cardiac dulness extends from 7 cm. to the right of the midsternal line in the fourth interspace to 14.5

TABLE 2.—Calorimetric Measurements in Case of Charles S.

Time	Right	Left	Time	Right	Notes
2:45	.....	30.65	3:00	30.95	
2:46	30.61	.....	3:01	30.99	Room temp. 28.3 C.
2:47	.....	30.68	3:02	31.005	
2:48	30.67	.....	3:03	31.02	
2:49	.....	30.73	3:04	31.035	3:04 L. hand put in water at 43 C.
2:50	30.73	.....	3:05	31.055	Room temp. 28.3 C.
2:51	.....	30.77	3:06	31.09	
2:52	30.76	.....	3:07	31.11	
2:53	.....	30.825	3:08	31.125	
2:54	30.825	.....	3:09	31.135	
2:55	.....	30.87	3:10	31.155	
2:56	30.88	.....	3:11	31.20	At 3:11 hand out of calorimeter.
2:57	30.905	.....	3:12½	.....	Temp. of L. is now 30.76 C.
2:58	30.92	.....	3:29	31.08	Room temp. 28.15 C.
2:59	30.94	.....			

Cooling of calorimeters, R., 0.12 degree in eighteen minutes, L., 0.11 C. degree in seventeen and one-half minutes. Volume of right hand in calorimeter 437 c.c., of left hand 410 c.c.

cm. to the left of the midsternal line in the fifth interspace. The first sound at the apex is very tympanitic in quality with a slightly roughened element in it. Second sound normal, no murmurs or adventitious sounds. The blood-vessels are sclerotic. Pulse regular in rate and rhythm. The ascent is slow, no dicrotism. Blood-pressure: systolic, 180; diastolic, 120. Some edema of the skin of the genitalia and of the ankles is present. The skin is cool and dry. He has been on cardiac diet and treated with atropin and nitroglycerin. Hemoglobin, 95 per cent. Diagnosis, arteriosclerosis and myocarditis. He was discharged "improved," July 19, 1911.

The blood-flow in the hand was examined July 13, 1911. Mouth temperature, 36.3 C.; pulse (sitting), 80. Hands put into bath at 2:32 p. m.; into calorimeters at 2:44. At 2:55 p. m. left hand put into water at 8.5 C. Cooling of calorimeters, R., 0.12 degrees in eighteen minutes; L., 0.11 degree in seventeen and one-half minutes. Volume of right hand in calorimeters, 437 c.c.; of left hand, 410 c.c.

The last case to be referred to under this heading is that of Fred S., a man 58 years old, with pronounced arteriosclerosis of the vessels of the limbs, mitral insufficiency and pulmonary emphysema. The initial flow was 5.76 grams per 100 c.c. per minute for the right hand and 5.80 grams for the left hand, with an average room temperature of 23.8 C. Immersion of the left hand in warm water caused no change in the flow in the right for three minutes, an unusually sluggish reaction, but for

TABLE 3.—CALORIMETRIC MEASUREMENTS IN CASE OF FRED S.

Time	Right	Left	Room Temp. C.	Time	Right	Room Temp. C.	Notes
2:32½	31.09	31.07	....	3:00	31.78	....	
2:35	31.06	31.05	23.4	3:01	31.81	....	
2:36	31.07	31.05	....	3:02	31.855	24.0	
2:37	31.08	31.06	23.6	3:03	31.895	....	At 3:03 left hand put in water at 8 C. He feels it very cold and withdraws hand intermittently .
2:38	31.095	31.07	....	3:04	31.91	....	
2:39	31.11	31.08	....	3:05	31.92	23.9	
2:40	31.125	31.10	24.0	3:06	31.925	....	
2:42	31.17	31.15	....	3:07	31.95	....	
2:43	31.195	31.165	.....	3:08	31.975	24.0	
2:44	31.22	31.185	.....	3:09	31.995	....	
2:45	31.25	31.22	23.8	3:10	32.02	....	
2:46	31.29	31.26	.....	3:11	32.04	24.0	
2:47	31.32	31.29	.....	3:12	32.06	....	At 3:12 left hand put in water at 38.5 C.
2:48	31.36	31.34	23.9	3:13	32.085	....	
2:49	31.395	31.37	....	3:14	32.11	....	
2:50	31.425	31.40	.....	3:15	32.13	24.2	
2:51	31.45	.....	23.9	3:16	32.165	....	
2:52	31.48	.....	.....	3:17	32.195	....	
2:53	31.505	.....	.....	3:18	32.22	24.2	
2:54	31.54	.....	24.0	3:19	32.24	....	
2:55	31.59	.....	.....	3:20	32.27	....	
2:56	31.62	.....	.....	3:21	32.295	....	
2:57	31.66	.....	23.9	3:22	32.32	....	At 3:22 hand out of calor.
2:58	31.70	.....	.....	3:23	.....	....	3:23 Temp. of L. is 31.00 C.
2:59	31.74	.....	24.0	3:45	32.02	....	3:45 Temp. of L. is 30.79 C.

the next ten minutes of immersion of the left hand in warm water the flow in the right rose to 7.69 grams per 100 c.c. per minute. Immersion of the left hand in cold water caused a reduction of the flow in the right to 3.65 grams per 100 c.c. per minute for the first three minutes, the flow then rising to 5.54 grams per 100 c.c. per minute for the next six minutes of immersion. When the left hand was again put into the warm water, there was only a small increase in the flow in the right (to 6.46 grams per 100 c.c. per minute for ten minutes' immersion).

Fred S., a laborer, aged 58, was admitted to the City Hospital April 27, 1913, with marked arteriosclerosis, pulmonary emphysema and mitral insufficiency.

He complains of shortness of breath (asthma) which began in October last. He has not been able to work since. He says the condition seems stationary, neither improving nor growing worse. When he gets chilled an attack is brought on. The point of maximal cardiac impulse is 5 cm. outside the left nipple line. The greatest breadth of cardiac dulness extends at the sixth rib to the anterior axillary line; right border, right sternal margin. Roughened first sound at apex followed by systolic murmur, which is lost as the base is approached. Pulse fairly regular rhythm and rate, fair volume. Liver enlarged. Urine contains no albumin. A few hyaline and finely granular casts. Hot baths seemed to have a good effect on him.

The blood-flow in the hands was examined May 8, 1913. Hands in bath at 2:22½ p. m., in calorimeters at 2:33½. At 2:50 p. m. left hand put into water at 43 C. (Table 3.)

Cooling of calorimeters, R., 0.30 degree in twenty-three minutes, L., 0.61 degree in fifty-five minutes. Pulse (sitting) 116. Volume of right hand 497 c.c., of left hand 484 c.c. Rectal temperature 37.4 C. Water equivalent of calorimeters with contents, R., 3,492, L., 3,482. Blood-pressure left arm, systolic 126, 63 (sound gone). Another observation. Systolic 122, 101 (sudden diminution of sound), 63 (sound gone).

II. AORTIC ANEURYSM

If we leave out of account John M., a dispensary case who came in with cold hands on a winter day and could not wait for sufficient time to warm up and whose measured flow (3.96 grams per 100 c.c. per minute for the right hand) is therefore certainly too low,[4] a point of some consequence comes out in Table 19 in which the results are summarized; that is, that for the age of the patient and the temperature of the room the hand flow in these cases of thoracic aneurysm are either of the normal order of magnitude or at least not so conspicuously deficient as in those cases dealt with in Paper X, in which the stress of the pathological change falls on the myocardium rather than on the blood-vessels.

John M., an iron-molder, aged 32 years, weight 140 pounds, height 5 feet, 3½ inches, was admitted at the dispensary suffering from aortic aneurysm as shown by the Roentgen ray. He complains of pain over the sternum. Systolic blood-pressure, right arm 155, left arm 150. Blood-flow in the hands examined Feb. 13, 1911. Pulse (sitting) 72. Mouth temperature 37.0 C. Room temperature 22.2 C. His hands were cold when he came in. Right hand in bath at 2:56 p. m., in calorimeter at 3:07 and out of calorimeters at 3:36. At 3:25 left hand put into water at 8.5 C.

It is not, of course, suggested that in some of the cases of aneurysm deterioration and disordered function of the myocardium or crippling of the valves may not be of graver import than the aneurysm itself. But so far as the aneurysm, or rather the underlying condition which has occasioned the aneurysm has not led to deterioration of the heart, there is no reason in the mechanics of the circulation why the blood-flow through

4. The fact that immersion of the left hand in cold water caused only a very slight diminution in the flow in the right while immersion of the left hand in warm water increased the flow decidedly (to 5.5 grams) indicates that the vasoconstriction produced by the exposure of the hand to the cold was initially considerable.

the hand should suffer. This is true even where the form of the pulse-wave may be greatly distorted by its passage through the aneurysmal sac. Of course, a sufficient degree of pressure of the sac on a subclavian artery or vein may diminish the flow in the corresponding hand. The great difference in the flow in the two hands in Paul K. may be accounted for by the pressure of the aneurysm on the left subclavian artery. The flow in his hands (for the last fifteen minutes in the calorimeters) was 7.64 grams for the right per 100 c.c. per minute and 5.9 grams for the left, with room temperature 22.6 C. (a ratio of 1.3:1). For the right hand this flow is not markedly subnormal for his age. The diagnosis was auricular fibrillation with mitral insufficiency (and stenosis), well compensated at the time of the blood-flow examination and aortic aneurysm. There

TABLE 4.—CALORIMETRIC MEASUREMENTS IN CASE OF JOHN M.

Time	Right	Time	Right	Time	Right
3:06	29.15	3:19	29.28	3:29	29.47
3:10*	29.13	3:20	29.30	3:30†	29.48
3:11	29.14	3:21	29.32	3:31	29.50
3:12	29.14	3:22	29.33	3:32	29.51
3:13	29.16	3:23	29.36	3:33	29.54
3:14	29.19	3:24	29.38	3:34‡	29.58
3:15	29.21	3:25	29.40	3:35	29.62
3:16	29.23	3:26	29.42	3:36	29.67
3:17	29.24	3:27	29.425	3:53	29.49
3:18	29.27	3:28	29.44	3:55	29.47

* Room 22.0 C. † At 3:30 left hand dried and wrapped up. ‡ Room 22.4 C. Volume of right hand 380 c.c.

is no evidence that the aneurysm, unless by its mechanical pressure, caused any change in the flow.

Paul K., a baker, 57 years old, was admitted to the City Hospital Feb. 21, 1912. There is a history of lues and of frequent acute attacks of articular rheumatism. A history of skin rash was obtained. He had gonorrhea ten or twelve times. His present illness began three weeks before admission. He had an attack of rheumatism at the time and began to be short of breath. His eyes have been swollen for three days. There is bulging over the heart. The thorax is barrel-shaped, and many dry râles can be heard. A loud systolic murmur can be heard over the apex, the heart is very irregular. Not all the heart-beats are felt at the wrist. Blood-pressure is 110 to 120. The Roentgen ray shows a marked prominence of the aortic arch on the left side and heart enlarged. He was put on digitalis. The pulse-rate, which was 132 on the morning after admission, fell rapidly. During March and April it varied between 75 and 90, his clinical condition improved and he was allowed to leave his bed and walk about the ward.

The blood-flow in the hands was examined April 24, 1912. Hands in bath at 1:32 p. m., in calorimeters at 1:42¾, and out of calorimeters at 2:06. (Table 5.)

Cooling of calorimeters in ten minutes, R., 0.13 degree; L., 0.12 degree. Volume of right hand 454 c.c., of left hand 451 c.c. He works mainly with his left hand although he is right-handed. Rectal temperature 38 C. Water equivalent of calorimeters with contents, R., 3,458; L., 3,456. After the examination it was noticed that the heart-rate was much increased (to 140 counted with a stethoscope) and that it was more irregular than before.

In Joseph C., a dispensary patient aged 50, with marked arteriosclerosis, a high systolic pressure (160 mm.) and distinctly dilated aortic arch, the flow was 6.69 grams for the right hand and 6.66 grams for the left, with room temperature 21.5 C. Considering the amount of arteriosclerosis and that in arteriosclerosis, as already stated, the hand flow has a tendency to be diminished, there is no evidence in this case that the presence of the aneurysm as such has reduced the flow.

Joseph C., an iron molder, aged 50, height 5 feet, 9½ inches, was admitted to the dispensary Jan. 30, 1911. He has been ill for six or seven years, during which time he has done no work. Six years ago he lost consciousness and after that his left arm was paralyzed. The grip of the left hand is weak, too weak, he says, to grasp objects; but on testing this the difference between the hands is not great. He gets very dizzy at times. There is much arteriosclerosis. He

TABLE 5.—Calorimetric Measurements in Case of Paul K.

Time	Right	Left	Room Temp. C.	Time	Right	Left	Room Temp. C.
1:42	31.13	31.29	....	1:56	31.40	31.465	....
1:44	31.07	31.21	....	1:57	31.45	31.50	....
1:45	31.08	31.21	22.8	1:58	31.49	31.525	22.6
1:46	31.095	31.225	....	1:59	31.51	31.53	....
1:47	31.11	31.23	....	2:00	31.56	31.58	....
1:48	31.13	31.25	22.8	2:01	31.605	31.61	....
1:49	31.165	31.29	....	2:02	31.65	31.635	....
1:50	31.19	31.31	....	2:03	31.70	31.67	....
1:51	31.21	31.32	....	2:04	31.75	31.71	22.6
1:52	31.25	31.35	....	2:05	31.795	31.75	....
1:53	31.29	31.38	22.9	2:06	31.83	31.76	....
1:54	31.32	31.41	....	2:16	31.70	31.64	....
1:55	31.37	31.43	....	....	.....	.....	....

suffers from pain in the head, also in the breast and in the left hand. The Roentgen ray shows a definite dilatation of the arch of the aorta. Wassermann reaction negative on Feb. 6, 1911.

The hand flow was examined on Jan. 31, 1911. Hands in bath at 2:52 p. m., in calorimeters at 3:05, and out of calorimeters at 3:20; 3,056 c.c. of water in each calorimeter. Pulse (sitting) 88. Mouth temperature 37.2 C. (Table 6.)

Cooling of calorimeters, R., 0.19 degree, and L. 0.16 degree in fourteen minutes. Volume of right hand 505 c.c., of left 480 c.c. Blood-pressure, systolic in right brachial 160, in left brachial 164.

In William B., a man aged 53, suffering from thoracic aneurysm, the flow in the right hand was 8.38 grams per 100 c.c. per minute and in the left 7.33 grams with room temperature 20.8 C. (for fourteen minutes

in calorimeters). The systolic pressure was very high (205 mm.). He was still taking nitroglycerin at the time of the blood-flow examination.

A second examination made at the dispensary nearly four weeks later showed a flow of 10.23 grams per 100 c.c. per minute for the right and 9.47 grams for the left hand, with room temperature 22.2 C. These flows are certainly not small for a man of his age and with sclerosed arteries. The inequality in the flow in the two hands is no greater than may be seen in normal persons, and it will be observed that the ratio between the flows in the two hands is the same at the two examinations (1:1.08).

TABLE 6.—CALORIMETRIC MEASUREMENTS IN CASE OF JOSEPH C.

Time	Right	Left	Room Temp. C.	Time	Right	Left	Room Temp. C.
3:04	29.13	29.12	20.3	3:16	29.55	29.51	....
3:06	29.11	29.10	....	3:17	29.61	29.53	21.5
3:07	29.14	29.11	....	3:18	29.67	29.62	....
3:08	29.18	29.12	21.5	3:19	29.72	29.67	....
3:09	29.20	29.14	....	3:20	29.78	29.71	....
3:10	29.25	29.19	....	3:32	29.61	29.58	....
3:11	29.29	29.22	....	3:34	29.59	29.55	....
3:12	29.34	29.31	....	....	.....	.....	....
3:13	29.39	29.33	21.4	....	.....	.....	....
3:14	29.45	29.39	....	....	.....	.....	....
3:15	29.51	29.43	....	....	.....	.....	....

TABLE 7.—CALORIMETRIC MEASUREMENTS IN CASE OF WILLIAM B. FIRST EXAMINATION

Time	Right	Left	Time	Right	Left
2:37	28.38	28.54	2:48	28.70	28.82
2:40	28.34	28.53	2:51	28.90	29.00
2:42	28.39	28.58	2:53	29.04	29.11
2:43	28.43	28.61	2:55	29.20	29.22
2:45	28.56	28.70	2:56	29.27	29.28
2:46	28.61	28.75	3:12	29.09	29.10

William B., a man aged 53, height 5 feet, 2 inches, weight 152 pounds, had an aneurysm of the thoracic aorta involving the innominate also to some extent. After being under observation at the dispensary for a considerable time he was admitted to Lakeside Hospital where he was under treatment for a month (digitalis and nitroglycerin). His hand flow was examined at the time of his discharge from the hospital. He felt much better than when he entered, on Dec. 22, 1910. He has no cough but there is a history of cyanosis, although there is no cyanosis now. He says his hands always feel rather cool, especially when his heart is bad.

Hands in bath at 2:26½ p. m., in calorimeters at 2:38½, and out of calorimeters at 2:56; 3,050 c.c. of water in each calorimeter. Room temperature 20.4 C.

Room temperature at end of observations 21.2 C. Volume of right hand in calorimeter 410 c.c., of left hand 390 c.c. Cooling of calorimeters in sixteen minutes 0.18 C. degree. Pulse (sitting) 84. Mouth temperature 37.1 C. Water equivalent of calorimeters with contents, R., 3,458; L., 3,442. (Table 7.)

The second examination was on Jan. 17, 1911. He says he feels about the same as at the first examination, only the shortness of breath on exertion is greater. Systolic blood-pressure 210. Hands put into bath at 3:09 p. m., in calorimeters at 3:21, and out of calorimeters at 3:37; 3,050 c.c. of water in each calorimeter. Room temperature 22.2 C.

TABLE 8.—Calorimetric Measurements in Case of William B. Second Examination

Time	Right	Left	Time	Right	Left
3:19	30.70	30.49	3:31	31.13	30.86
3:23	30.69	30.48	3:32	31.19	30.91
3:25	30.79	30.57	3:33	31.23	30.94
3:26	30.86	30.62	3:34	31.29	31.00
3:27	30.92	30.67	3:35	31.34	31.07
3:28	30.97	30.71	3:36	31.39	31.11
3:29	31.02	30.77	3:37	31.44	31.16
3:30	31.07	30.81	4:00	31.12	30.85

Cooling of calorimeters in twenty-three minutes, R., 0.32 C. degree; L., 0.31 C. degree. Volume of right hand in calorimeter 435 c.c., of left 410 c.c. Mouth temperature 36.9 C. Water equivalent of calorimeters with contents, R., 3,478; L., 3,458. Pulse (sitting) 88.

In Richard B, a man aged 38, with a large aortic aneurysm extending, as shown by the Roentgen-ray, to a considerable distance to the right of the sternum, there was a decided deficiency in the flow of the left hand as compared with the right (10.91 grams per 100 c.c. per minute for the right, 8.15 grams for the left, a ratio of 1.33:1). For the room temperature of 28 C., 10.9 grams is not a large flow, but neither is it decidedly small. The vasomotor reflexes from the right to the left hand were normal in direction, and not strikingly deficient in intensity, showing that the chief afferent path in the vasomotor reaction to warmth or cold (the nerves of warmth and cold sensation) have not appreciably suffered by stagnation of the circulation due to the pressure of the aneurysm on the right subclavian vein. Now, when pressure is maintained on the arm at a level somewhat below the systolic arterial pressure by means of the cuff of a sphygmomanometer the warmth sensation suffers early, so that it is impossible to appreciate the contact of a warm tube, while the sensation of pain is at this stage less affected. As has been pointed out in Paper X, a certain amount of venous distention, provided that the arterial pressure is correspondingly increased, may even be associated with an

increased flow, because of the increased cross-section of the smaller vessels. So far as the arterial path to the right arm in Richard B. is concerned, the conditions would favor a greater flow in the right hand, for as shown at the necropsy the aneurysm provided a much wider entrance than normal for the blood going to the right subclavian and possibly a reservoir which helped to maintain the flow during diastole.

Richard B., a colored gardener, aged 38, height 5 feet, 7½ inches, was admitted to Lakeside Hospital May 22, 1911, with an aneurysm diagnosed as aortic. He complains of hoarseness during the past two weeks and vomiting during the past week, also cough. He admits lues. There is a tender pulsating mass in the suprasternal notch with fulness in the right supraclavicular region. Heart 3 cm. to left of nipple. A distant systolic murmur at the supraclavicular notch. The aortic second is slightly accentuated. There is distinct diastolic shock, unilateral (right) distention of veins, and clubbing of fingers. The Roentgen ray shows an enormous shadow upward and to the right. The patient continued in the hospital until his death, Aug. 22, 1911. There was no right radial pulse for the last week of life. Blood examination on May 23, 1911, showed hemoglobin 95 per cent., leukocytes 5,000. June 1 the Wassermann test was strongly positive. The necropsy showed a saccular, spherical aneurysm of the innominate artery 3 to 4 inches in diameter. The arch of the aorta was not involved except for the somewhat enlarged ring at the beginning of the innominate opening into the sac.

Hand flow examined May 23, 1911. Pulse (sitting) 100. Mouth temperature 37.1 C. Hands put into bath at 2:44 p. m., into calorimeters at 2:54½. At 3:08 p. m. right hand put into water at 8.1 C.

TABLE 9.—Calorimetric Measurements in Case of Richard B.

Time	Right	Left	Room Temp. C.	Time	Left	Room Temp. C.	Notes
2:54	30.43	30.38	....	3:13	31.225	....	
2:55	30.475	30.42	27.7	3:14	31.28	....	
2:56	30.53	30.43	....	3:15	31.325	28.0	
2:57	30.595	30.46	....	3:16	31.36	....	At 3:16 put right hand in water at 43 C.
2:58	30.695	30.525	....	3:17	31.38	....	
2:59	30.78	30.60	....	3:18	31.415	....	
3:00	30.86	30.63	....	3:19	31.46	....	
3:01	30.935	30.69	27.9	3:20	31.525	28.3	
3:02	31.02	30.74	....	3:21	31.58	....	
3:03	31.12	30.825	....	3:22	31.625	28.2	
3:04	31.20	.....	....	3:23	31.67	....	
3:05	31.295	30.93	....	3:24	31.725	....	
3:06	31.34	30.98	....	3:25	31.80	....	
3:07	31.395	31.02	....	3:26	31.85	28.2	Hand out of calorimeter.
3:08	31.46	31.035	....	3:41	31.75	28.0	Temp. of R. is now 31.27 C.
3:09	.....	31.07	28.0				
3:10	.....	31.10	....				
3:11	.....	31.125	....				
3:12	.....	31.155	....				

Cooling of calorimeters, R., 0.19 degree in thirty-three minutes; L., 0.10 C. degree in fifteen minutes. Volume of right hand 495 c.c., of left 437 c.c. There is an obvious difference in the size of the two hands. He is right-handed.

The next case, that of Samuel S., a man 31 years old, illustrates the fact that even very marked differences in the form of the radial pulse on the two sides, differences perfectly evident both to the finger and on the sphygmogram, may be associated with a comparatively small difference in the flow in the two hands. The right radial showed a typical pulse of aortic insufficiency with abrupt ascent to a sharp apex and abrupt fall, while the left radial gave a sphymogram with a rounded apex. The blood-pressure in the two brachials was also quite different, the systolic pressure being 157 in the right and 118 in the left. The physical signs of aortic insufficiency were present.

The blood-flow measurement showed a difference no greater than may be seen in normal persons between the two hands (11.11 grams per 100 c.c. per minute for the right and 10.32 grams for the left, with room temperature 22.2 C.), a perfectly normal flow for a man of this age. The conclusion was drawn that the aneurysm in distorting the form of the pulse-wave did not interfere materially with the free passage of blood through the left subclavian artery. The man died the same night about five hours after the examination by a rupture of the aneurysm into the left bronchus. It is worthy of remark that five hours before his death the blood-flow in his hands, which no doubt in this case was an index of the output of his heart, was quite normal. He was to die, not because his heart was failing, but because the wall of the aneurysmal sac had been thinned to the bursting point. A very different tale would have been told by the hand flow in a case of impending heart-failure as in Stephen F., or Albert H. (Paper X), or Anthony P.

Samuel S., a colored music teacher, aged 31, was admitted to the City Hospital Dec. 7, 1912, with thoracic aneurysm. He is a well-developed man 5 feet, 10½ inches tall, weight 160 pounds. He has had spells of intense dyspnea for the past six weeks. He has been continually getting worse to the present time, is much cyanosed, and suffers much from cough which he has had from the age of 16. He has smoked cigarettes to excess, and also has used a good deal of alcohol. He denies lues although he admits the possibility of it.

The left pupil is smaller than the right and does not react to light or accommodation. There is diffuse pulsation of the lower third of the neck. Tracheal tug is present. The left side of the thorax moves less than the right. The percussion note is dull over the entire left side [compression of the left lung by the aneurism(?)]. Over the left lung the breath-sounds are very faint, distant and bronchial in character. The cardiac dulness at the level of the second rib extends three fingers' breadth to the right of the right sternal border and the same distance to the left of the left sternal border. In the fourth interpsace it extends from two fingers' breadth to the right of the right sternal border to the left midaxillary line. The apex beat is visible and palpable in the sixth interspace, two fingers' breadth outside the nipple line. Systolic and presystolic thrills are palpable over the apex. Loud booming systolic and diastolic murmurs are heard at apex. Loud presystolic murmur followed by systolic murmur is heard over aortic and pulmonic areas. The sternum rises fully a half-inch with each systole. The radial pulse is regular, rhythmical, asymmetrical, the sphygmogram of the left radial having a rounded apex, and that of the right a sharp (aortic insufficiency) apex. The pulse is synchronous in the two radials and

of good volume and tension. The difference in the form of the pulse-wave is very evident to the finger.

The blood-flow in the hand was measured Dec. 10, 1912. Hands in bath at 1:50 p. m., in calorimeters at 1:59½, and out of calorimeters at 2:13 p. m.

TABLE 10.—Calorimetric Measurements in Case of Samuel S.

Time	Right	Left	Room Temp. C.	Time	Right	Left	Room Temp. C.
1:59	31.40	31.43	....	2:07	32.11	32.13	22.25
2:00	31.435	31.49	22.4	2:08	32.195	32.20	....
2:01	31.55	31.58	22.4	2:09	32.27	32.265	22.2
2:02	31.64	31.68	....	2:10	32.35	32.36	22.1
2:03	31.74	31.78	22.3	2:11	32.395	32.42	....
2:04	31.85	31.88	....	2:12	32.47	32.465	22.3
2:05	31.94	31.95	....	2:13	32.53	32.53	....
2:06	32.01	32.055	22.3	2:41	32.17	32.17	....

Cooling of calorimeters in twenty-eight minutes, 0.36 degree. Volume of right hand 580 c.c., of left hand 603 c.c. Water equivalent of calorimeters with contents, R., 3,559; L., 3,577. Rectal temperature 38.4 C. Blood-pressure left arm, systolic 118, 58 (sound suddenly diminished); right arm, systolic 157, 61 (sound abruptly diminished). Another observation on the right arm gave identical results. Three days previously, according to another observer, the pressures were 160 (systolic), 45 (change of sound) in the right, and 130, 55 in the left brachial.

A necropsy was performed by Dr. A. A. Johnson, Pathologist at the City Hospital, to whom I am indebted for the following details. Heart, left ventricle dilated moderately, average thickness 16 to 18 mm. Other cavities normal. The segments of all the valves except the aortic are normal and the orifices are not dilated. The aortic ring in the formaldehyd-preserved specimen measured 8 cm. The aortic valve segments are slightly thickened and curled along their free edges. The openings of the coronary arteries are free and of the usual size. The aorta immediately above the aortic ring is smooth and free from thickening. Opening from the anterior aspect of the transverse arch is a large sacular aneurysm adherent to the sternum. The sac is oval, 10 by 8 cm. in diameter. The walls are thin (2 to 4 mm.) and are quite free from fibrin. The opening from the aorta into the sac measures 5 by 3.5 cm. The innominate opens by a somewhat dilated mouth posterior and external to the opening into the aneurysm and situated opposite the middle of the latter opening. The left common carotid and subclavian arteries come off from the aorta about 1 cm. beyond the upper border of the opening between the aorta and the aneurysm. Rupture in the sac on the inferior surface has irregular edges and measures 3 by 1 mm., leading into the left bronchus 1 cm. from the bifurcation.

It will be seen that there was no stenosis of the orifice of the left subclavian, but its origin was situated distal to the orifice of the aneurysmal sac, while the origin of the innominate was directly opposite the center of that orifice. The direction of the opening of the innominate was such that the blood coming from the heart would pass straight into it. all the more as the opening of the innominate was somewhat dilated. This would account for the abrupt rise in the sphygmographic curve and

the high maximum pressure in the right arm. The sudden fall in the sphygmogram if partly due to actual regurgitation through the aortic orifice, was probably due still more to the effect of rapid expansion of the thin aneurysmal sac, which after closure of the aortic valve would still go on yielding and so exert a suction action, especially on the orifice of the innominate placed directly opposite it. On the other hand, the action of the large aneurysmal sac lying proximal to the left subclavian would be to damp the pulse-wave in that artery and delay the arrival of the crest. This would not necessarily affect the total flow, the pulsatory element in which is measured not by the absolute height of the crest, but by the area enclosed between the sphygmogram and the line of diastolic pressure.[5]

The last case included in the group of aneurysms (that of John McL.) is the only one which shows a notably small hand flow. It is placed in this group merely to emphasize the point that in drawing any general deductions from such measurements the proper classification of the cases is of great importance.

John McL., a plasterer, aged 50, height 5 feet, 11½ inches, was admitted to the City Hospital July 6, 1912, with general edema (in face, hands, arms, legs and feet), also cyanosis in the lips and fingers. He complains of "kidney trouble." He looks anemic. The heart shows an impure first sound at the apex. The aortic second is greatly accentuated, the blood-pressure is high (systolic 200). The abdomen is distended with free fluid. Albumin and casts were present in the urine all along. On July 11 about 7 liters of fluid was removed from the abdomen. There was no detectable fluid in the thoracic cavity. A diagnosis of hepatic cirrhosis was made. Aug. 19, 7,080 c.c. of fluid was removed from the abdomen.

On Aug. 27, 1912, the hand and foot flows were estimated and found exceedingly small (0.41 gram per 100 c.c. per minute for the right hand and 0.57 gram for the left hand, with room temperature 22.2 C.; 0.17 gram per 100 c.c. per minute for the right foot and 0.14 gram for the left foot, with room temperature 21.2 C.).

Aug. 28, 1912, 1,965 c.c. of liquid was taken from the abdominal cavity at 1:30 p. m., and the hand and foot flows were again measured. They were larger than on the previous day, but still quite small (1.11 grams per 100 c.c. per minute for a period of seventeen minutes for the right hand and 1.18 grams for the left hand, with room temperature 21.1 C.; 0.66 gram per 100 c.c. per minute for the right foot for the last ten minutes of a period beginning twenty minutes after insertion of the feet into the calorimeters, and 0.65 gram for the left foot). The ratio between the flows in the feet and hands (1:1.75) is near enough the normal to permit the conclusion that the small flow in both has a common cause. The presence of an aneurysm of the aorta was not

5. Paper V of this series, THE ARCHIVES INT. MED., 1912, ix, 706.

diagnosed during life. The small blood-flow in the hands has certainly nothing to do with the existence of the aneurysm. It is without doubt dependent on the cardiorenal, or cardiorenalhepatic affection, that is to say, on such factors as the vasoconstriction of which the high blood-pressure is one expression, on the coincident anemia, and on the crippling of the heart muscle. The small flow in the feet may also depend in part on the mechanical hindrance imposed by the fluid in the peritoneal cavity or in the connective tissue of the legs. But if this were an important factor the ratio of hand to foot flow would be disturbed.

The patient died Dec. 27, 1912. The necropsy showed general peritonitis, aneurysm of the descending thoracic aorta, cloudy swelling and parenchymatous change in the kidneys. The pericardium was adherent to the left pleura, but not to the epicardium. The heart was pale (weight 300 grams), with some dilatation and hypertrophy of the left ventricle. The mitral orifice was dilated and the edges of the mitral valve were thickened and puckered. The edges of the aortic valve were thickened and slightly curled. The sinuses of Valsalva were dilated. Many sclerotic plaques were about the coronary openings. The right coronary vessels were not patent. The left coronary vessels were free but sclerosed. The aortic ring was much dilated, 10.5 cm. in circumference. The whole intima of the aorta was very much thickened. Here and there were areas of sclerosis. The openings of the large vessels were dilated. Eight centimeters beyond this there was an opening on the left posterior wall of the aorta 2 cm. in diameter, opening into a sac 4 cm. in diameter. The body of the fifth thoracic vertebra had been slightly eroded. Beyond this the thoracic aorta was much thickened in irregular areas. About 1,000 c.c. of straw-colored fluid was in the left pleural cavity. The abdomen contained about 10 liters of cloudy fluid with many shreds of fibrin. The visceral and the parietal peritoneum was dark in color and covered with exudate. The intestines were adherent. The liver showed no scarring; weight 1,660 grams. In the kidneys the cortex was not sharply differentiated from the medulla. The whole kidney was dark in appearance, with cloudy swelling to parenchymatous change. For these details I am obliged to Dr. A. A. Johnson.

III. MISCELLANEOUS VASCULAR CONDITIONS INCLUDING VASCULAR NEUROSES

Among these are three cases of Raynaud's disease.

Margaret H., aged 15, suffering from Raynaud's disease in the fingers, had a flow in the right hand of 5.33 grams per 100 c.c. of hand per minute and 6.67 grams in the left, with room temperature 23.6 C. The protocol has already been published.[6] The considerable inequality in the flow in the two hands is probably due to a functional vasomotor difference. When there are no anatomical reasons for such a difference in flow it either disappears at subsequent examinations or is reversed as regards the two hands. In cases in which a marked difference is obtained in the two hands in several examinations, the deficiency being always in the same hand and the ratio of the flows in the two hands not changing greatly, there is reason to believe, in the absence of obvious anatomical differences, that the difference in flow may depend on pathological

6. Heart, 1911, iii, 79.

changes in the thorax or in the arm or hand itself which have not yet definitely disclosed themselves by other symptoms.

The flow in Margaret H. is distinctly subnormal, which corresponds with the fact that her hands were cold to the touch, although she did not have an attack during the examination. The relatively small flow may be related to the tendency to dyspnea which she states has existed for some years. The most striking result of the examination was the intense reflex vasoconstriction produced in the left hand when the right was put into cold water, the flow being reduced from 6.67 grams to 2.97 grams per 100 c.c. per minute. Although intense, the vasoconstriction was transient, lasting only for two minutes and then giving place to distinct vasodilatation, the flow increasing to 8.28 grams in the left hand, while the right continued in the cold water. Immersion of the right hand in warm water produced no further increase, but rather a decrease. The vasomotor mechanism of the hand seems, therefore, to respond with abnormal readiness to vasoconstrictor influences and with less than normal readiness to vasodilator influences. The short duration of the intense crossed reflex vasoconstriction relates the case to those of early peripheral neuritis,[7] and it is of interest, in this connection, that neuritic changes may be associated with the Raynaud's syndrome.

The second case of Raynaud's disease was that of Mrs. W. G., aged 28, who was seen April 24, 1911. About one and one-half years previously, after a sleigh ride, she noticed that the fingers of both hands became cold, "white like those of a dead person" and numb. Sometimes the fingers became purple. All the phalanges were involved. She feels sure they were not frost-bitten. Since that time she has had many attacks, each lasting usually five to ten minutes, but occasionally almost an hour. The attack generally begins in the thumb. Putting the hands into cold water does not necessarily bring on an attack. A cold full bath is more likely to do so. The thumbs are apt to become numb if she holds anything in her hands for any length of time. Long before this condition came on her arms and hands used to go to sleep a great deal even when she was not lying on them. Also for as long as she can remember small injuries to the skin of the hands or arms healed badly. She pointed out the scar of a small burn on the left hand which was still raised above the surface although otherwise healed. It was a most trifling burn she said. Even a little scratch leaves a scar. She showed various marks of old injuries on the arm. She has been fighting the condition by going out to walk without gloves, continuing to walk with her hands exposed even when they became numb, and thought that some time she improved it in this way, her hands afterwards becoming warm. Her general method has been to immerse them in warm water on coming in. The attacks are apt to be induced by a damp day, even if not cold. The hands to-day feel markedly cold to the touch, not only the fingers, but the hands midway to the wrists. The day was not cold and she was wearing kid gloves. Her arms are mottled with purple spots. She has no trouble in the feet, nose, or elsewhere. Her general health is satisfactory, but there is some shortness of breath on exertion. She is fat and well nourished, and the arms and hands are quite plump. She suffers much from nervousness.

7. Paper XII of this series.

The blood-flow in the hands was examined April 24, 1911. Pulse 108 (sitting). Mouth temperature 37.55 C. Hands put into bath at 10:10 a. m. At 10:15 it was seen that the left arm was getting purple; the right arm remained unchanged. She said the left hand felt colder than the right, and that the left arm and hand felt as if swollen. Hands put into calorimeters at 10:20 a. m.

TABLE 11.—Calorimetric Measurements in Case of Mrs. W. G.

Time	Right	Left	Notes	Time	Right	Notes
10:19	30.125	30.13		10:43	30.57	
10:24	30.11	30.13		10:44	30.60	
10:25	30.12	30.15	Room temp. 25.3 C.	10:45	30.62	
10:27	30.17	30.19		10:46	30.67	Room temp. 24.9 C.
10:28	30.20	30.22		10:47	30.70	At 10:47 left hand put in water at 12 C.
10:29	30.22	30.24				
10:30	30.25	30.26		10:48	30.71	
10:31	30.26	30.26		10:49	30.72	
10:32	30.27	30.27		10:50	30.73	
10:33	30.29	30.31		10:51	30.75	
10:34	30.31	30.32	At 10.34 put left hand in water at 43 C.	10:52	30.77	
				10:53	30.79	
10:35	30.33	.....				
10:36	30.36	.....	Room temp. 27 C. Face and ears red. She says the left hand feels swollen greatly.	10:54	30.81	At 10:54 dried and wrapped left hand. It is red and is not specially cold to touch. Room temp. 27.4 C.
10:37	30.38	.....		10:55	30.83	
10:38	30.40	.....		10:56	30.86	
10:39	30.42	.....	Room Temp. 25.3 C.	10:57	30.89	
10:40	30.47	.....		10:58	30.92	Hand out of calorimeter at 10:58.
10:41	30.50	.....				
10:42	30.54	.....		10:59	.....	10:59 temp. of L. is 30.30 C.
				11:24	30.74	11:24 temp. of L. is 30.06 C. Room temp. 25.8 C.

Cooling of calorimeters, R., 0.18 C. degree in twenty-six minutes; L., 0.14 degree in twenty-five minutes. Volume of right hand 391 c.c., of left hand 375 c.c. Water equivalent of calorimeters with contents, R., 3,408; L., 3,395. The left hand may be swollen a little, as her ring, which had been removed, now goes on with difficulty, yet the subjective feeling of extreme swelling which she had is obviously no index of the actual swelling.

The small initial flow in this patient (3.62 grams per 100 c.c. per minute for the right hand and 3.44 grams for the left, with the relatively high room temperature of 25.3 C.) is considerably more striking than in the case of Margaret H. This agrees well with the longer duration and greater development of the condition in Mrs. W. G. Immersion of the left hand in warm water caused a fair and persistent increase in the flow in the right (to 5.16 grams), although the absolute flow was still small. Subsequent immersion of the left hand in cold water caused a strong vasoconstriction in the right hand, diminution of the flow to 2.46 grams per 100 c.c. per minute for the first three minutes and 3.93 grams for the last four minutes. Although the temperature of the cold water was

purposely made higher than usual (12 C. instead of 8 C.), she complained greatly of the cold and withdrew her hand intermittently. This was seen also in the other cases, and the hypersensitiveness of the afferent receptors to cold as gauged by the sensation elicited has its parallel in their hypersensitiveness to cold as components of the reflex vasomotor arc.

The third case of Raynaud's disease (Mrs. C. B.) illustrates the condition when it has existed for a long time and has gone on to gangrene. The age of the patient was 29. Not only was the local condition more severe than in the last case, but the general physical condition was worse. The hand flow for a period of sixteen minutes before the vasomotor reaction was tested was 3.23 grams per 100 c.c. per minute for the right hand and 4.29 grams for the left. For the last six minutes of this period the flows worked out at 3.65 grams and 3.80 grams for the right and left hands, respectively. The reflex reaction to warmth was fair (the flow in the right hand increasing for the first two minutes of immersion of the left in warm water to 6.55 grams per 100 c.c. per minute), but it was transient, and for the last ten minutes of the period the flow was only 4.30 grams per 100 c.c. per minute. The reflex reaction to cold was also anomalous, possibly because of the existence at this stage of the disease of considerable anatomical changes in the vessel.

Mrs. C. B., aged 24, was admitted to the Lakeside Dispensary June 1, 1908, suffering from Raynaud's disease. She was treated with nitroglycerin without benefit. April 23, 1913, on account of coughs, sweats, and general weakness she was referred to the Medical Department of the Dispensary when the following data were elicited. Both hands are affected. The condition was first noted one day in winter seven years ago, the fingers becoming cold and pale and then getting purple. The attacks have been growing steadily worse, especially in the last five years. Sometimes small grangrenous areas form quickly at the ends of the fingers and quickly recover, but always with some loss of substance, so that the terminal phalanges of several of the fingers have lost a considerable proportion of their length and most of them have lost their nails. On all the fingers the nails are atrophic. She has considerable pain in the fingers when they "break out." She has had pain and marked stiffness in the right wrist and right forearm for about two years, and she uses the right hand but little on account of the stiffness of the wrist. The skin of the right forearm is somewhat stretched and distinctly glossy in comparison with that of the left. She cannot bear to put her hands into cold water. She has no such symptoms in her feet, nose or elsewhere. Some years ago she had a sore on her ankle, due, as she thinks, to a habit of kicking the ankle with the other foot. The sore refused to heal and she was put in hospital for several weeks, during which time her general health and strength improved greatly, and the sore also improved, although it took a long time before it was healed. She is pale and thin. There is some pigmentation of the skin and some dulness at the apex of the left lung. The radial pulse is small.

Examination of the blood-flow in the hands was made May 9, 1913. The marked pallor with some lividity and the "stone cold" feeling of the hands, especially of the fingers, was striking, although she was wearing gloves. The day was rather chilly. The terminal phalanx of one finger of the right hand had a small sore on the point of it which she said caused her some pain. Pulse (sitting) 112, weak. Hands in bath at 3:15 p. m., in calorimeters at 3:27. Mouth temperature, 37.35 C.

TABLE 12.—Calorimetric Measurements in Case of Mrs. C. B.

Time	Right	Left	Room Temp. C.	Time	Right	Notes
3:26	31.18	31.17	.....	3:53	31.135	3:52 left hand put in water at 43 C.
3:28	31.09	31.13	.....	3:54	31.155	Room 22.8.
3:29	31.09	31.13	21.3	3:55	31.16	
3:30	31.08	31.12	.....	3:56	31.17	Room temp. 22.9 C.
3:31	31.08	31.12	21.5	3:57	31.175	
3:32	31.08	31.11	.....	3:58	31.18	
3:33	31.085	31.11	.....	3:59	31.195	Room temp. 22.95 C.
3:34	31.08	31.11	21.8	4:00	31.20	
3:35	31.08	31.11	.....	4:01	31.20	
3:36	31.08	31.115	.....	4:02	31.205	
3:37	31.085	31.12	22.0	4:03	31.21	
3:38	31.085	31.12	.....	4:04	31.225	At 4:04 left hand put in water at 12 C.
3:39	31.085	31.125	.....	4:05	31.24	She feels it very cold. Left hand taken out of water intermittently.
3:40	31.09	31.14	.....	4:06	31.25	
3:41	31.085	31.15	22.1	4:07	31.255	Room temp. 22.7 C.
3:42	31.09	31.16	.....	4:08	31.26	The cold water is now at 13.5 C. The left hand is red. She says if it had not first been in warm water it would have been purple in the cold water. To the touch the left wrist is warm (vasodilatation), much warmer than the right.
3:43	31.09	31.17	.....	4:09	31.27	
3:44	31.09	31.18	.....	4:10	31.28	
3:45	31.09	31.195	.....	4:11	31.295	
3:46	31.095	31.20	22.0	4:12	31.305	
3:47	31.10	31.205	.....	4:13	31.34	4:13 hand out calorimeter.
3:48	31.10	31.21	.....	4:43	30.96	4:43 temp. L. 30.58 C.
3:49	31.105	31.215	.....			
3:50	31.115	31.22	22.6			
3:51	31.12	31.225	.....			
3:52	31.12	31.23	.....			

Cooling of calorimeters, R., 0.38 C. in 30 minutes; L., 0.65 C. in 51 minutes. Volume of right hand 273 c.c., of left 282 c.c. She is right handed. Water equivalent of calorimeters with contents, R., 3.313; L., 3.320.

It may be noted that in these three cases of Raynaud's disease there was evidence that a coexisting or preexisting general feebleness of the circulation conspired with the local condition in producing the symptoms. The blood-flow measurements suggested that if the general circulatory conditions had been normal the local tendency to vasoconstriction might not have sufficed to reduce the flow to the level at which the symptoms became obtrusive. This was the suggestion merely derived

from the three cases studied and it is not claimed that it has a general application. At any rate since hand flows habitually as small or smaller are often encountered in cases which never develop Raynaud's disease, the primary factor must be a tendency, whatever its essential cause, to the local diminution in the flow.

In a case of "dead fingers" in an elderly man affecting only the right hand, the flows were among the smallest observed in the whole investigation, and this not only in the hand obviously affected, but also in the other. The patient had been sitting in a well-warmed room for an hour before the examination, yet the calculated flow for the right hand was only 0.09 gram per 100 c.c. per minute and for the left 0.25 gram, with room temperature 20.5 C. A fair reflex vasodilatation was obtained in the right hand on the immersion of the left in warm water, the flow increasing to 0.5 gram, still of course a very small flow, but relatively a great increase. The dead condition of the right hand is therefore probably not due to anatomical change in the vessels, but to a vasoconstriction.

The next case in this group is that of Maurice T., a man 41 years old, who had lost a leg through obliterative arteritis.

Maurice T., a tailor's cutter, height 5 feet 7 inches, weight about 175 pounds, a well-built man, apparently in perfect health. He was seen April 17, 1911. About eight years ago in winter he began to feel the toes of the left foot cold. The little toe was first affected; gradually the same condition came on in the other toes. After awhile the toes became painful also. The whole foot became involved by degrees. Then cramp-like pains began to trouble him in the calf of the left leg. The trouble was worse in winter. Small nodules on the foot and toes became gangrenous. Eventually, four years ago, the left leg had to be amputated above the knee. The arteries were found extensively affected. He never had any pain above the knee. About six months ago he noticed that the little toe of his right foot was cold. Later on it began to pain him. Now the whole right foot is cold to the touch and the pulse in the dorsalis pedis poor. The little toe has a raw surface where it is contact with the next toe and the nail is apparently going to come off. He knows of no injury to it. The pain in the foot is so great that while he is in bed he cannot sleep. When he stands the pain disappears, but he has pain also when he walks. The arch of the right foot is broken down and he has to wear a support in his shoe. There is hyperidrosis of the foot in spite of the fact that it is cold. The flow in the hands was examined April 17, 1911. Mouth temperature 36.5 C. Hands put into bath at 10:05 a. m. and into calorimeters at 10:18. Pulse (sitting), 92.

At this time the foot calorimeters had not been constructed so that only an examination of the flow in the hands was made. This was done with the idea that some information of possible value for a prognosis in regard to the leg condition might be obtained, (1) from the absolute amount of the hand flow as an index of the flow in the periphery in general, (2) from the nature of the reflex vasomotor response as an index of the existence of a tendency to vasomotor spasm, a functional condition which there is good reason to believe may underlie or at least precede in certain cases the development of obliterative arteritis. The flow in the

right hand was 4.87 grams per 100 c.c. per minute and in the left 6.67 grams, with room temperature 25.7 C., a distinctly subnormal flow. Immersion of the right hand in cold water caused one of the most intense reflex vasoconstrictions in the contra-lateral hand which has been witnessed in the whole course of these observations, the flow declining to 2.59 grams per 100 c.c. per minute for the first five minutes and only reaching 5.59 grams after a further immersion in the cold water of four

TABLE 13.—CALORIMETRIC MEASUREMENTS IN CASE OF MAURICE T.

Time.	Right	Left	Notes.	Time.	Left	Notes.
10:17½	29.57	29.58		10:38	30.24	Room temperature 25.5 C.
10:20	29.58	29.60				
10:21	29.60	29.62		10:39	30.29	At 10:39 put right hand in water at 43°.
10:22	29.62	29.65	Room temperature 25.7 C.			
10:23	29.66	29.70		10:40	30.31	
10:24	29.70	29.76		10:41	30.34	Room temperature 25.0 C.
10:25	29.74	29.82				
10:26	29.78	29.86	Room temperature 25.6 C.	10:42	30.38	Room temperature 25.1 C.
10:27	29.81	29.92		10:43	30.45	
10:28	29.85	29.97	Room temperature, 26 C. Ears red.	10:44	30.52	Room temperature 25.2 C.
				10:45	30.60	Ears still red.
10:29	29.89	30.02		10:46	30.65	
10:29½	....	....	Right hand put in water at 9 C.	10:47	30.72	Room temperature 25.2 C.
10:30	....	30.08		10:48	30.80	At 10:48 dried and wrapped right hand.
10:31	....	30 09				
10:32	....	30.11	The cold water is now 10.6 C.	10:49	30.82	
10:33	....	30.12	He feels the cold water extremely.	10:50	30.88	Room temperature 25.2 C.
10:34	....	30.14	Room temperature 26. 6 C.	10:51	30.94	
				10:52	31.00	Room temperature 25.3. C.
10:35	....	30.15				
10:36	....	30.19		10:53	31.07	
10:37	....	30.22		10:54	31.13	Hand out of cal.
				11:09	....	Temp. of room 29.65 C.
				11:09½	31.01	

Volume of right hand, 470 c.c.; of left hand, 466 c.c. He is right-handed.

minutes. The reflex to warmth is also well marked, the flow in the left hand eventually rising to 10.96 grams. The general indication of the observations as regards the question propounded was that a vasomotor instability with a tendency to excessive vasoconstriction in the extremities might be the basis for the symptoms in the foot. The inequality in the flows in the two hands corroborates the idea of some degree of vasomotor abnormality. Anatomical changes in the nerves were of course not excluded. Unfortunately, the man soon after removed to a distant part

of the country, and up to the present it has not proved possible to learn the further history of the case.

In the case of Anthony P., a man 36 years old, with gangrene of the fingers and toes, the question arose whether the condition depended on a vasomotor neurosis.

Anthony P., a Polish laborer, aged 36 years, height 5 feet 8 inches, weight 150 pounds, was admitted to City Hospital, Oct. 9, 1912, complaining of sore fingers and toes. He froze his feet last winter. There is no history of frost-bite of the fingers. He had numbness of the fingers a year ago. Owing to the condition of his fingers he was forced to quit work about three weeks before admission. On admission the right hand showed beginning gangrene at the tip of the thumb, loss of the distal half of the nail of the middle finger, the remainder being undermined and loss of the entire nail of the ring-finger. The left hand showed purplish discoloration of the thumb, the nail being absent, loss of the entire nail of the index finger except the root, and gangrene about the base of the nail of the ring-finger.

A blood-flow examination of the hands was made on Oct. 11, 1912, with a very warm day (room temperature 27.8 C.). The flow came out only 1.22 grams for the right hand, and 1.12 grams for the left per 100 c.c. per minute, or allowing for the swelling of the left hand, 1.27 grams. The pulse-rate was 120 (sitting). The conclusion drawn was that if the small flow depended solely on the local conditions it was unlikely that the constriction of the vessels was purely functional, since the high room temperature would have caused vasodilatation. He was in such poor condition that a sufficient examination of the vasomotor reflexes was postponed. In the meantime cyanosis of the face and dilatation of the neck veins became prominent and physical examination revealed the signs of serious involvement of the heart (mitral stenosis, aortic insufficiency and signs of impairment of the myocardium). It was no longer possible to draw deductions from the small hand flow, and the slight response to the high external temperature as to the condition of the local circulation, since the state of the heart itself was sufficient to account for the small flow even provided that the cutaneous vasodilatation was the maximum possible with so small a driving power. Also fever (his rectal temperature was 39.65 C.) is associated with cutaneous vasoconstriction and a small hand and foot flow.[8] Had the feet been normal, a comparison of the hand and foot flow might still have yielded information, since the cardiac insufficiency might be expected to diminish the flow in both without affecting the normal ratio between the hand and foot flow, whereas a vascular constriction (vasomotor or anatomical) confined to the hands would be revealed by a disturbance of this ratio in favor of the feet. The local conditions, however, soon became a matter of indifference in view of the serious involvement of the heart. The patient died October 12.

8. Stewart, G. N.: Paper VIII, Jour. Exper. Med., 1913, xviii, 372.

The necropsy showed the following: Left lung: infarct in the lower lobe 5 cm. in diameter. Right lung: infarct in upper lobe 2½ cm. in diameter, and in lower lobe 5 cm. in diameter. Heart engorged on the right side and left side dilated; heart-muscle dark and flabby. The pericardium contains 100 c.c. of fluid. Aortic valve, left cusp hardened and shortened. Mitral admits tip of index-finger. Vegetations about the valves. Tricuspid, edges of valves thickened, recent vegetations. Liver, 1,800 grams, with chronic passive congestion. Spleen, weight 140 grams, chronic passive congestion. The kidneys show much arterial thickening. Blood-vessels: radial, practically no arterial thickening. The fingers show stasis of blood in the vessels. A cross-section of the index-finger shows much perivascular infiltration, but no thickening of the vessel walls. Pathological diagnosis, septic endocarditis.—(From Dr. Johnson's report.)

In a case of diabetic gangrene of the feet in a man 63 years old, the flow with a room temperature of 25.6 C. was 1.39 grams for the right foot and 1.83 grams for the left, or allowing for the swelling of the latter 1.95 grams. An interesting point is that the flow in the foot in which the gangrene had made most progress was greater than in the other foot. There is scarcely any question that this is due to inflammatory reaction. The diminished flow in the toes actually involved, if the flow is diminished there, would not show in a measurement of the flow through the whole foot. The sum of the flows in the feet, although subnormal, is not strikingly deficient, and the ratio of foot to hand flow (1:1.85) falls within the normal range. The hand flow was measured on the day preceding the observations on the foot and came out 2.65 grams for the right hand and 3.32 grams for the left per 100 c.c. per minute, with room temperature 22.0 C. In hospital cases presenting no rapid clinical changes, our experience has shown that such observations can safely be compared. The fact that the flow both in the hands and feet is decidedly below the normal agrees with the well-known feebleness of the cutaneous circulation in diabetes, and is probably in part due to cardiac changes. The vasomotor reflex, when warm water is applied to the contralateral hand, is very poor. Whether this indicates the beginning of pathological changes in the hand arteries also cannot of course be definitely stated. It is in any case interesting to correlate the enfeeblement of this vasomotor reflex with the enfeeblement or disappearance of skeletal reflexes such as the knee-jerk, which is known to occur in long-standing cases.

James P., a teamster 63 years old, was admitted to the City Hospital, Aug. 16, 1911, with a small ulcer on the inner surface of the left great toe and a scar in the same position on the right great toe. He came for treatment for his foot. It was found that the urine contained 4 per cent. of sugar with a trace of albumin. He was put on a diabetic diet. The blood-count showed erythrocytes 4,890,000, leukocytes 7,800, hemoglobin 90 per cent. April 28, 1912, the percentage of sugar in the urine was reduced to 0.8. April 11, 1912, when the blood-flow in the feet was examined there were small necrotic areas on the great and second toes of the right foot. The distal half of the left foot was bluish-red and the foot considerably swollen. The toes of the right foot were also bluish-red. He says that sometimes the left foot is hot, but when the flow in the feet was examined this was not the case.

Feet in bath at 1:21 p. m.; in calorimeters at 1:33, and out of calorimeters at 2:00; 3,740 c.c. of water in each calorimeter.[9]

Mouth temperature 37.45 C. Pulse (sitting), 68.

TABLE 14.—Calorimetric Measurements in Case of James P.

Time.	Right	Left	Room Temp. C.	Time.	Right	Left	Room Temp. C.
1:37	30.82	30.76	....	1:51	30.995	30.97	....
1:38	30.82	30.80	25.9	1:52	30.98	31.00	....
1:39	30.86	30.82	....	1:53	31.01	31.01	25.4
1:40	30.875	30.83	....	1:54	31.02	31.025	....
1:41	30.885	30.84	....	1:55	31.03	31.04	....
1:42	30.885	30.855	25.8	1:56	31.035	31.05	....
1:43	30.905	30.87	....	1:57	31.05	31.06	....
1:44	30.92	30.88	....	1:58	31.05	31.08	....
1:45	30.935	30.915	....	1:59	31.06	31.10	....
1:46	30.94	30.92	....	2:00	31.02	31.09	25.4
1:47	30.95	30.935	....	2:28	30.76	.....	....
1:48	30.96	30.95	25.2	2:29	.....	30.75	....
1:49	30.975	30.96	....	2:32	30.71*	.....	....
1:50	30.98	30.96	....	2:33	.....	30.72*	....

* After better stirring.

Cooling of calorimeters, R., 0.31 degree in thirty-two minutes, L., 0.37 degree in thirty-three minutes. Volume of right foot in calorimeter 1,090 c.c. of left foot 1,175 c.c. Rectal temperature (taken by himself and doubtless too low as compared with mouth temperature), 37.5 C. Water equivalent of calorimeters with contents, R., 4,760, L., 4,820.

The next case is remarkable for the fact that the flow in the hands per 100 c.c. per minute was very much less than in the feet, a quite unusual circumstance.

K., a laborer aged 50, was admitted April 15, 1912, to the City Hospital. He was much cyanosed on admission, and when the flow in the hands was first examined next day the cyanosis was still well marked in the face, nose, ears and hands, although less pronounced than on admission. There is gangrene of the toes on left foot. The pulse can be felt in the dorsalis pedis. Blood-pressure 140 systolic, 100 diastolic. He had some cough and râles of every description were heard all over the lungs. The fingers were markedly clubbed. The heart-sounds were weak. A soft systolic murmur and reduplicated second sound were heard over the left sternal border, more marked at the base. The heart was enlarged. The pulse at the wrist is not strong, but by no means so weak as to suggest that the blood-flow in the hands would be found so small as is actually the case.

April 16 the flow in the right hand came out only 0.27 gram per 100 c.c. per minute and in the left hand 0.28 gram, for a period of eighteen minutes in the calorimeters, with room temperature 23.8 C. Immersion of the left hand in warm water caused no increase whatever in the flow in the right. The very small flow in the hands agreed with the diagnosis

9. For the details of measurements of foot-flow see Paper VII of this series, Jour. Exper. Med., 1913, xviii, 354.

of myocarditis. It is possible also that a factor in the small flow was nervousness, as this was the first examination, and the patient was a foreigner, speaking no English. Still the effect of nervousness on the flow usually passes away more quickly.

April 18 he was again examined. The cyanosis was now much diminished and the radial pulse was stronger. The flow in the right hand was 1.05 grams and in the left 1.43 grams per 100 c.c. per minute, with room temperature 22.5 C., still quite a small flow, although much greater than at the last examination. In the right foot the flow was 2.15 grams per 100 c.c. per minute, and in the left foot 3.42 grams. Immersion of the right foot in cold water was accompanied by a reduction in the flow in the left foot to 2.68 grams, which was only slightly increased (to 2.87 grams) when the right foot was put into warm water.

Feet in bath at 2:07 p. m., in calorimeters 2:19 p. m. Patient sitting. 3.740 c.c. water in each calorimeter. At 2:45 p. m. the right foot was put into water at 10.5 C.

TABLE 15.—Calorimetric Measurements in Case of K.

Time	Right	Left	Room Temp. C.	Time	Left	Notes
2:22	29.99	29.93	.....	2:46	30.605	
2:23	30.00	29.95	20.5	2:47	30.625	
2:24	30.02	29.99	.....	2:48	30.635	Room temp. 20.7 C.
2:25	30.03	30.05	.....	2:49	30.655	
2:26	30.045	30.08	.....	2:50	30.665	
2:27	30.07	30.125	.....	2:51	30.70	
2:28	30.08	30.14	20.4	2:52	30.715	
2:29	30.09	30.175	.....	2:53	30.725	
2:30	30.10	30.22	.....	2:54	30.745	At 2:54 right foot put in
2:31	30.11	30.245	.....			water at 42.8 C.
2:32	30.125	30.275	.....	2:55	30.76	Room temp., 21.0 C.
2:33	30.135	30.30	.....	2:56	30.775	Right big toe painful.
2:34	30.145	30.33	.....	2:57	30.80	
2:35	30.17	30.35	20.1	2:58	30.825	
2:36	30.19	30.38	.....	2:59	30.835	
2:38	30.21	30.42	.....	3:00	30.85	
2:39	30.22	30.46	.....	3:01	30.87	
2:40	30.23	30.47	.....	3:02	30.90	Room temp. 21.3 C.
2:41	30.23	30.50	.....	3:03	30.925	
2:42	30.235	30.52	.....	3:04	30.935	
2:43	30.24	30.535	.....	3:05	30.94	Foot out of calorimeter.
2:44	30.26	30.56	.....	3:17	30.75	Room temp. 20.9 C.
2:45	30.275	30.595	.....	3:18	.....	Temp. of R. is now 29.79 C.

Cooling of calorimeters, R., 0.485 C. in thirty-three minutes; L., 0.19 C. in twelve minutes. Rectal temperature 37.6 C. Volume of right foot in calorimeter 950 c.c., of left 985 c.c. Water equivalent of calorimeters with contents, R., 4,652; L., 4,679.

The question whether the gangrene was going to spread was what chiefly interested the physician in charge of the case. The fact that the

flow in the feet was so good was interpreted as favorable for the prognosis, although of course it was impossible to say to what extent the flow in the toes themselves had been interfered with. The left foot was somewhat swollen, as shown by the volume measurement. Yet it was only a little over 3 per cent. larger than the right. The greater flow in the left was probably related to a certain degree of inflammatory reaction. The foot soon began to heal up. But on April 25 the temperature, which had hitherto never surpassed 100 F., began to rise, reaching 104.2 F. on April 27. Then it fell and remained below 100 F. till his death on May 3.

The existence of a relatively good flow in the feet associated with a poor flow in the hands demands some further remark. It is probable that the immediate cause of the lesion in the left foot was exposure to cold. If so, it is likely that the already existing feebleness of the circulation and the poor response of the vasomotor regulatory mechanism were contributing causes. Very likely both feet suffered injury, although it only went on to gangrene in the left. The flow in both feet may, therefore, have been increased by the reaction to the injury caused by the freezing, and this in spite of the feeble driving-power of the heart. Thus the flow in the feet may have been brought up by the accident of the inflammation approximately to the normal flow, while that in the hands fell far below the normal.

Miss W., aged 25, weight 128 pounds, has lost fourteen pounds since her illness began. She first noticed pain in the ankles with some swelling in March, 1908. The pain increases as the day goes on, and at about 7 p. m. it is worse but on lying down she gets relief. In the morning her eyes are swollen. The wrists also swell when she works much with her arms. There is some shortness of breath. She must lie down some part of the day if she desires to sit up at night, even to 9 o'clock. An eruption consisting of raised red spots comes out periodically on the legs, also, though much less copiously, on the arms. There is burning pain and itching in the spots. In a day they get brownish, and in two days have disappeared to a great extent, except that a brown stain persists for weeks. Several spots 2 cm. in diameter which had come out the previous day on her legs were examined. The reddish color did not diminish on pressure. From her description the spots resemble urticaria at first and then hemorrhage takes place into the wheals. On the trunk also are spots which she describes as wheals, which itch and are painful, but the most purpuric-looking spots are on the legs. On the front of each tibia is a spot with a blackish crust from which she says some matter came out, but generally no liquid escapes from them. Her feet and toes are cold and so are her hands, especially the terminal phalanges of the fingers. Her nails grow irregularly, probably more quickly than before, and are ridged. There is distinct defect of sensation in the toes and the adjacent parts of the foot. A pin-prick is scarcely felt, and then only as contact. She does not feel any tickling sensation when the sole is stroked and there is no plantar reflex. Good knee-jerks can be obtained. There is a long reddish scar on the back of one finger of the left hand. She says she had "blood-poisoning" some time ago in a small wound caused by a tack and healing did not occur for eighteen weeks. The heart-sounds are clear and unaccompanied by murmurs. The urine contains no albumin, casts or sugar. Her eye-sight is not affected.

The blood-flow in the hands was examined May 9, 1911. Pulse 88. Mouth temperature 37.6 C. Hands put into bath at 10:14½ p. m., and into calorimeters at 10:24.

TABLE 16.—Calorimetric Measurements in Case of Miss W.

Time.	Right	Left	Notes.	Time.	Right	Notes.
10:23	30.13	30.08	Room temperature 24.0 C.	10:43	30.195	Room temperature 24.3 C.
10:25	30.11	30.06		10:44	30.20	
10:26	30.10	30.05		10:45	30.215	At 10:45 left hand put in water at 9.1 C.
10:27	30.11	30.05				
10:28	30.09	30.04				
10:29	30.10	30.04	Room temperature 24.1 C.	10:46	30.215	She feels left hand very uncomfortable.
10:30	30.10	30.04				
10:31	30.10	30 03		10:47	30.22	
10:32	30.12	30.04		10:48	30.225	Room temperature 24.3 C.
10:33	30 12	30.035				
10:34	30.125	30.04		10:49	30.225	
10:35	30.13	30.045		10:50	30.23	
10:36	30.145	30.06		10:51	30.23	
10:37	30.16	30.06	At 10:37 left hand put in water at 43 C.	10:52	30.235	Room temperature 24.4 C. At 10:52 dried and wrapped left hand. It is pretty red.
10:38	30.165	.....	Room temperature 24.3 C.			
10:39	30.17	.....	Left hand is fairly red and the veins of fair size.	10:53	30.235	
				10:54	30.24	
				10:55	30.245	Room temperature 24.5 C.
10:40	30.17	.....				
10:41	30.18	.....		10:56	30.245	Hand out of calorimeter. Feels pins needles in it.
10:42	30.185	.....				
				10:56½	.....	Temperature of
				11:26	30.03	L., 29.91 (at 10:56½).

Cooling of calorimeters, R., 0.215 C. in thirty minutes, L., 0.15 C. in nineteen and one-half minutes. Volume of right hand in calorimeter 292 c.c. of left 298 c.c. She is right-handed.

May 25, 1911, the physician in charge of the case reported that since the time of the blood-flow examination she had had rather severe intestinal symptoms and that her temperature rose to 103 F. He gave her calcium chlorid and alkalies. She is now much better than for a while and the spots have disappeared.

The flow in the right hand was 2.16 grams per 100 c.c. per minute, in the left 1.64 grams, with room temperature 24.1 C., a very poor flow for her age. Reflex vasodilatation is absent, or quite feeble, the flow in the right hand only rising to 2.38 grams when the left was immersed in warm water. Scraping the skin with a blunt point caused no local vasodilatation. Although the initial flow was so small a further reflex vasoconstriction, reducing the flow in the right hand to 1.72 grams, was obtained when the left hand was put into cold water. No doubt the

coexisting anemia is a factor in reducing the flow through the hands. The toxemia (rheumatic perhaps) on which the purpura depends may also depress the flow more directly, either by enfeebling the heart or by vasoconstriction. The conclusion deduced from the blood-flow measurements was that the feeble cutaneous flow might be a factor in the condition, and that so far as this went, the indication was that such measures should be adopted as would increase the flow in the skin, including the treatment of such anemia as was present.[10]

The last case to be described in this paper is that of Charles H.

Charles H., a clerk 42 years old, height 5 feet 10½ inches, weight 140 pounds, was admitted to the City Hospital Nov. 23, 1912, with edema of the right foot and leg. The swelling began a month ago, commencing at the toes with inflammation, he says, resembling erysipelas. A little over a year ago he accidentally injured the right lower leg by a blow with a hammer about midway between knee and ankle over the tibia. It healed at the time. Afterward a sore opened from which matter was discharged. He entered a hospital, where the leg soon healed. He has pain

TABLE 17.—Calorimetric Measurements in Case of Charles H. First Examination

Time	Right	Left	Room Temp. C.	Time	Right	Left	Room Temp. C.
3:26	31.43	31.43	....	3:51	31.15	31.165	....
3:29	31.36	31.31	22.7	3:53	31.135	31.18	23.0
3:31	31.33	31.26	....	3:55	31.125	31.185	23.1
3:33	31.305	31.24	22.8	3:57	31.12	31.19	23.15
3:35	31.28	31.23	23.1	3:59	31.105	31.21	23.1
3:37	31.26	31.195	23.15	4:01	31.09	31.225	....
3:39	31.235	31.18	....	4:03	31.08	31.24	23.0
3:41	31.22	31.17	23.1	4:05	31.07	31.24	....
3:43	31.20	31.16	....	4:07	31.06	31.245	23.0
3:45	31.18	31.155	23.1	4:10	31.01	31.18	....
3:47	31.17	31.155	23.0	4:21	30.865	31.04	....
3:49	31.16	31.15	....	....	.....	.....	....

as soon as he commences to walk. When he stands up for a few minutes the foot becomes very blue and the small blood-vessels distend. The swelling comes on with great rapidity (in ten or fifteen minutes) when he goes about on his feet, and disappears completely when he lies down for a little while. There are no varicose veins. He does not appreciate warmth or cold, or contact (blunt-point) on the dorsum of the right foot or on the lower third of the right leg. A pin-prick is felt as dull contact. There is no anesthesia above the level of the scar due to the injury, except for a small tongue just above and continuous with the scar. The right sole is very sensitive. It began to be hypersensitive just after the foot commenced to swell. When he awakes in the morning he has a burning sensation about the toe-nails of the right foot. This disappears when he walks about a little. When he stood up it was seen that his right leg became very red in the anesthetic region. On pressure the red color disappeared. He says that when he first came into the hospital the white spot produced on pressure remained for some time, but now it quickly becomes red again. When he first came into the hospital

10. Paper VI of this series, Jour. Exper. Med., 1913, xviii, 13.

the red color which came on when standing soon became pale blue, whereas after a fortnight in the hospital it remains red. The legs, however, continue to be cold to the touch. He would not know his leg and foot were becoming red if he did not see it. He says he cannot keep the right foot warm. When the left foot is sweating under the bedclothes and he puts his hand down to feel the right foot it is always cold. He looks anemic and feels pretty weak, though his appetite is good. He has lost nearly 30 pounds in weight since he had malaria in Texas three years ago. The knee-jerks and ankle-jerks are somewhat exaggerated, but there is no patellar or ankle clonus. Examination of the heart and lungs is negative in result and the heart sounds are clear though very weak and distant. The pulse is regular.

The flow in the feet was examined Dec. 5, 1912, the patient sitting. Feet in bath at 3:15 p. m., in calorimeters at 3:28, and out of calorimeters at 4:07; 2,775 c.c. of water in each calorimeter. (Table 17.)

Cooling of calorimeters in eleven minutes, R., 0.145 degree; L., 0.14 degree. Volume of right foot 961 c.c., of left 924 c.c. He has small feet (No. 7 shoes). Rectal temperature 37.6 C. Pulse (sitting) 100. Water equivalent of calorimeters with contents, R., 3,696, L., 3,668.

Second examination Dec. 10, 1912. Pulse (sitting) 96. Hands in bath at 3:14 p. m., in calorimeters at 3:27. At 3:40 right hand put into water at 42.8 C.

When taken out of the cold water the right hand was distinctly redder than the left. Cooling of calorimeters, R., 0.505 C. in forty-one minutes; L., 0.19 C. degree in sixteen minutes. Volume of right hand in calorimeter 367 c.c., of left hand 355 c.c. He is right-handed. Rectal temperature 37.35 C. Blood-pressure in right arm, systolic 108, 88 (sudden change in sound), 81 (sound gone). The left arm, systolic 108. (Table 18.)

TABLE 18.—Calorimetric Measurements in Case of Charles H. Second Examination

Time	Right	Left	Room Temp. C.	Time	Left	Room Temp. C.	Notes
3:26	31.89	31.85	21.9	3:47	31.90	22.5	
3:28	31.85	31.81	22.0	3:48	31.91	22.4	
3:29	31.85	31.81	22.15	3:49	31.92	....	
3:30	31.85	31.81	22.15	3:50	31.925	22.8	
3:31	31.855	31.82	....	3:51	31.93	....	
3:32	31.86	31.825	22.4	3:52	31.935	22.9	
3:33	31.865	31.83	22.6	3:53	31.94	....	
3:34	31.87	31.84	22.8	3:54	31.945	22.7	
3:35	31.875	31.84	....	3:55	31.955	....	At 3:55 right hand put in water at 8.6 C.
3:36	31.88	31.85	22.6	3:56	31.955	....	
3:37	31.88	31.855	22.5	3:57	31.955	....	
3:38	31.895	31.86	....	3:58	31.955	....	The right hand is red. He feels the water very cold.
3:39	31.895	31.86	....	3:59	31.95	23.7	
3:40	31.895	31.865	....	4:00	31.945	....	
3:41	.....	31.875	23.0	4:01	31.94	23.5	
3:42	.....	31.875	....	4:02	31.94	....	The right hand does not now feel cold at all. Still red.
3:43	.....	31.875	22.8	4:03	31.94	....	
3:44	.....	31.88	....	4:04	31.935	23.7	
3:45	.....	31.885	22.7	4:05	31.93	....	Hand out of calor. at 4:05.
3:46	.....	31.89	....	4:21	31.74	....	4:21 temp. of R. is 31.39 C.

The distribution of the anesthesia and the history of the case seem to indicate that a nerve injury was the starting point of the vascular condition. The flow in the right foot was 0.51 gram per 100 c.c. per minute and in the left 1.35 grams, with room temperature 23.0 C. The flow in the right hand was 3.43 grams, and in the left hand 3.71 grams per 100 c.c. per minute, with room temperature 22.6 C. While, then, the flow in the hands is much below the normal, which corresponds well with the rather feeble action of the heart and with the patient's anemic condition, the flow in the left foot preserves a ratio to that in the hands not far from normal. The flow in the right foot, however, is distinctly poor, even in proportion to the poor hand flow. The cause accordingly of the small flow in the right foot is not a central one, but is local. This agrees well with the patient's observation that he cannot keep the right foot warm, and that the red color which it first exhibits when he stands becomes pale blue. The blood-flow measurement corroborates the conclusion that in the right foot the blood is stagnating in the capillaries of the engorged area.

SUMMARY

1. In cases with marked arteriosclerosis the blood-flow in the hands is smaller and the contralateral vasomotor reflexes less pronounced than normal.

2. In cases of thoracic aneurysm the flow in the hands may be normal. Where it is subnormal there is no evidence, apart from a unilateral deficiency in some cases due to the pressure of the aneurysm, that the existence of the aneurysm as such has any influence in diminishing the flow, the diminished flow being associated with coexisting pathological conditions (myocardial changes, valvular lesions).

3. A great difference in the form of the pulse-wave in the two radials associated with thoracic aneurysm does not necessarily indicate a difference in the flow through the hands. The hand flows may be identical when the sphygmograms from the radials on the two sides are very different.

4. In three cases of Raynaud's disease the hand flows were subnormal even in the absence of an actual attack, in the more advanced cases very much below the normal.

5. An abnormally great contralateral reflex vasoconstriction to cold seems to characterize the earlier stages of Raynaud's malady. When it has existed for a long time and gangrene has occurred, the vasomotor reactions indicate the existence of anatomical changes in the vessels, which do not permit vasomotor reflexes as intense as at first.

TABLE 19.—SUMMARY OF CALORIMETRIC

Case	Age	Date	Pulse Rate	Blood Pressure	Temperature (C.) of				Volume of Part in c.c	
					Room	Art Blood	Calorimeters			
							Right	Left	Right	Left
Chas. S....	69	7/ 8/13	80	180, (120)	28.3	36.3	30.70	30.75	437	410
					28.3	....	30.96	.....	.....	.....
Fred S....	58	5/ 8/13	116	122, (101)* 63	28.3	....	31.12	.....	.....	.....
					23.8	36.9	31.28	31.25	497	484
					23.9	....	31.47	.....	.....	.....
					23.9	....	31.70	.....	.....	.....
					23.9	....	31.91	.....	.....	.....
					24.0	....	31.99	.....	.....	.....
					24.2	....	32.19	.....	.....	.....
Paul K...	57	4/24/12	100	115	22.8	37.5	31.14	31.27	454	451
					22.6	....	31.52	31.54	.....	.....
Joseph C..	50	1/31/11	88	160	21.5	37.2	29.49	29.43	505	480
Wm. B....	53	12/22/10	84	205	20.8	37.1	28.81	28.97	410	390
		1/17/11	88	210	22.2	36.9	31.07	30.82	435	410
Richard B.	38	5/23/11	100	.....	27.9	37.1	31.03	30.75	495	437
					28.0	....	.....	31.09	.....	.....
					28.0	....	.....	31.26	.....	.....
					28.1	....	.....	31.41	.....	.....
					28.2	....	.....	31.66	.....	.....
Sam S....	31	12/10/12	124	L.,118,(58) R.,157,(61)	22 2	37.9	31.98	32.01	580	603
John McL..	50	8/27/12	....	156, 83	22.2	36.15	30.73	30.72	498	475
					21.2	....	30.53	30.44	1,697	1,712
		8/28/12	97	.....	21.1	36.7	30.63	30.59	507	454
					20.4	....	30.87	30.76	1,780	1,749
Mrs. W. G.	28	4/24/11	108	.....	25.3	37.55	30.22	30 24	391	375
					25.1	....	30.51	.....	.....	.....
					25.0	....	30.73	.....	.....	.....
					....	....	30.77	.....	.....	.....
					2.66	....	30.87	.....	.....	.....
Mrs. C. B..	29	5/ 9/13	112	.....	21.7	37.35	31.09	31.10	273	282
					22.3	....	31.10	31.27	.....	.....
					22.3	....	31.11	31.21	.....	.....
					22.8	....	31.14	.....	.....	.....
					22.9	....	31.19	.....	.....	.....
					22.8	....	31.24	.....	.....	.....
					22.7	....	31.25	.....	.....	.....
					22.7	....	31.30	.....	.....	.....
Maurice T.	41	4/17/11	92	.....	25.7	36.5	29.84	29.76	470	466
					26.3	....	.....	30.11	.....	.....
					26.6	....	.....	30.22	.....	.....
					25.0	....	.....	30.34	.....	.....
					25.2	....	.....	30.59	.....	.....
Anthony P.	36	10/11/12	120	.....	27.8	39.15	31.90	31.92	406	460
James P...	63	4/10/12	....	.....	22.0	37.05	30.51	30.59	438	414
					22.0	....	.....	30.78	.....	.....
		4/11/12	68	.....	25.6	37.35	30.92	30.93	1,090	1,175
K.	50	4/16/12	116	140, 100	23.8	36.7	30.55	30.65	352	310
					24.0	....	30.44	.....	.....	.....
		4/18/12	....	.....	22.5	37.1	29.55	29.72	313	304
					20.3	37.0	30.13	30.26	950	985
					20.7	....	.....	30.67	.....	.....
					21.1	....	.....	30.84	.....	.....
Miss W...	25	5/ 9/11	88	.....	24.1	37.6	30.13	30.05	292	298
					24.3	....	30.19	.....	.....	.....
					24.3	....	30.23	.....	.....	.....
					24.4	....	30.24	.....	.....	.....
Chas. H....	42	12/ 5/12	100	.....	23.0	37.0	31.11	31.20	961	924
		12/10/12	96	108, (88) 81	22.6	36.8	31.87	31.84	367	355
					22.9	....	.....	31.87	.....	.....
					22.7	....	.....	31.92	.....	.....
					23.6	....	.....	31.93	.....	.....

* As in the other papers, the numbers in brackets in this column give the pressure at which the sound had disappeared.

The condensed results of three of the cases referred to in this paper (Meyer, G., J. O'R. and this series (Heart, 1911, iii, 33).

Heat Given Off in Gram-Calories			Blood-Flow in Gm. Per Min.		Flow Per 100 c.c. of Part Per Min.		Notes
Right	Left	In Mins.	Right	Left	Right	Left	
930	822	8	23.07	20.57	5.25	5.01	Arteriosclerosis.
723	.....	8	18.81	.....	4.30	.....	Left hand in water at 8.5 C.
724	.....	7	22.18	.....	5.08	.....	Left hand in water at 43 C.
1,449	1,428	10	28.64	28.08	5.76	5.80	Arteriosclerosis.
410	.....	3	27.96	.....	5.62	.....	Left hand in water at 43 C.
1,790	.....	10	38.24	.....	7.69	.....	Left hand still in water at 43 C.
244	.....	3	18.18	.....	3.65	.....	Left hand in water at 8c.
731	.....	6	27.57	.....	5.54	.....	Left hand still in water at 8 c.
1,362	.....	10	32.13	.....	6.46	.....	Left hand in water at 38.5 C.
795	656	7	19.84	16.71	4.37	3.70	Aneurysm first seven minutes.
2,801	2,143	15	34.69	26.63	7.64	5.90	Last fifteen minutes in calorimeters.
2,580	2,460	11	33.81	31.09	6.69	6.66	Aortic aneurysm.
3,581	2,927	14	34.36	28.59	8.38	7.33	Thoracic aneurysm.
3,269	2,974	14	44.50	38.82	10.23	9.47	
3,247	2,239	11	54.03	35.62	10.91	8.15	Innominate aneurysm.
....	517	4	.....	23.89	.....	5.47	Right hand in water at 8.1 C.
....	792	4	.....	37.63	.....	8.61	Right hand still in water at 8.1 C.
....	413	3	.....	26.91	.....	6.16	Right hand in water at 4.3 C.
....	1,516	7	.....	44.23	.....	10.12	
4,466	4,292	13	64.47	62.28	11.11	10.32	Aortic aneurysm.
157	208	14	2.07	2.73	0.41	0.57	Cardio-renal disease with aneurysm.
114	100	8	2.81	2.43	0.17	0.14	Feet.
525	501	17	5.65	5.36	1.11	1.18	
616	608	10	11.74	11.37	0.66	0.65	Feet.
842	754	9	14.18	12.93	3.62	3.44	Raynaud's disease.
1,534	.....	12	20.18	.....	5.16	.....	Left hand in water at 43 C.
170	.....	3	9.23	.....	2.46	.....	Left hand in water at 12 C.
375	.....	4	15.36	.....	3.93	.....	Left hand still in water at 12 C.
477	.....	4	19.83	.....	5.07	.....	Left hand dried and wrapped.
298	299	8	6.61	6.64	2.42	2.35	Raynaud's disease first eight minutes.
795	1,079	16	8.83	12.12	3.23	4.29	Next sixteen minutes in calorimeters.
392	415	7	9.97	10.73	3.65	3.80	Last seven minutes of the sixteen.
200	.....	2	17.89	.....	6.55	.....	Left hand in water at 43 C.
651	.....	10	11.74	.....	4.30	.....	Left hand still in water at 43 C.
167	.....	2	15.18	.....	5.56	.....	Left hand in water at 12 C.
118	.....	2	10.74	.....	3.93	.....	Left hand still in water at 12 C.
477	.....	5	1,752	.....	6.41	.....	Left hand still in water at 12 C.
1,491	1,111	8	31.09	22.89	6.67	4.87	Arteritis in leg.
....	347	5	.....	12.07	.....	2.59	Right hand in water at 9 C.
....	589	4	.....	26.06	.....	5.59	Right hand still in water at 9 C.
....	382	3	.....	22.97	.....	4.93	Right hand in water at 43 C.
....	1,630	6	.....	51.09	.....	10.96	Right hand still in water at 43 C.
324	336	10	4.96	5.16	1.22	1.12	Gangrene of fingers; septic endocarditis.
1,033	1,199	15	11.59	13.75	2.65	3.32	Diabetes.
....	1,027	11	.....	16.54	.....	3.99	Right hand in water at 43 C.
2,023	2,858	23	15.19	21.50	1.39	1.83	Feet (left gangrenous).
95	87	18	0.95	0.88	0.27	0.28	Myocarditis; cyanosis. (Congenital heart?)
44	.....	10	0.78	.....	0.22	.....	Left hand in water at 41.8 C.
335	434	15	3.29	4.35	1.05	1.43	Cyanosis much less.
2,912	4,712	23	20.47	33.77	2.15	3.42	Feet (left toes gangrenous).
....	1,357	9	.....	26.46	.....	2.68	Right foot in water at 10.5 C.
....	1,726	11	.....	28.30	.....	2.87	Right foot in water at 42 C.
467	300	R 11 L 9	6.32	4.90	2.16	1.64	Purpura.
372	.....	8	6.97	.....	2.38	.....	Left hand in water at 43 C.
234	.....	7	5.04	.....	1.72	.....	Left hand in water at 9.1 C.
133	.....	4	5.04	.....	1.72	.....	Left hand dried and wrapped.
474	1,192	18	4.96	12.67	0.51	1.37	Feet (edema of right).
559	588	10	12.59	13.17	3.43	3.71	Hands.
....	210	4	.....	11.83	.....	3.33	Right hand in water at 42.8 C.
....	700	11	.....	14.49	.....	4.08	Right hand still in water at 42.8 C.
....	321	10	.....	7.32	.....	2.06	Right hand in water at 8.6 C.

the abrupt change in the sound occurred; the smaller number following is the pressure at which

Margaret H.) are omitted from the table, having already been given in Table II, Paper I, of

CHRONIC GASTRIC ULCER AND ITS RELATION TO GASTRIC CARCINOMA*

REVIEW OF SIX HUNDRED EIGHTY-FOUR SPECIMENS †

WILLIAM CARPENTER MacCARTY, M.D.
AND
ALBERT COMPTON BRODERS, M.D.
MAYO CLINIC
ROCHESTER, MINN.

The discussion which is going on in the literature over the question of whether or not gastric carcinoma develops on chronic gastric ulcer and regarding the percentage of chronic ulcers which "become" carcinomatous, is, it seems to us, a discussion which is not only dealing with an unanswerable question with our present methods of investigation, but is also doing a great deal of unnecessary harm to mankind by preventing rational treatment. Viewed from the standpoint of feasibility of scientific investigation, such discussion seems superfluous for the following reasons: 1. No one has seen a chronic gastric ulcer in the process of development. 2. No one has been able to witness the stages of reaction to irritation through which the tissues of the gastric wall pass during the formation of ulcer or carcinoma. 3. No one has ever recognized carcinoma in the process of development anywhere in man or other animal. 4. Even the most skilful cellular pathologist cannot point out the line of demarcation between simple hyperplastic cells of the mucosa of a simple chronic ulcer and malignant hyperplastic cells of a chronic ulcer which is associated with carcinoma. 5. No one has experimentally produced a carcinoma.

With these fundamental limitations of our knowledge, one can only view the conditions as they occur. The known facts which are seen in the specimens herewith presented are the following:

1. Single and multiple chronic ulcerations occur in the stomach (Figs. 1, 2, 13 and 18).

2. They vary widely in size (diameter and depth), as in Figures 1, 2, 5, 6, 8, 9, 10, 11, 13. 14, 18, 19, 29 and 33.

---

* Read before the Southern Minnesota Medical Society, Mankato, Aug. 5, 1913, and before the Kentucky Medical Society, Sept. 2, 1913.

† This number includes 218 specimens previously reported by MacCarty at the meeting of the American Medical Association, June, 1909, and published in Surgery, Gynecology and Obstetrics, May, 1910.

3. The characteristics of simple chronic gastric ulcers consist of excavations in the wall of the stomach, and these excavations may have the mucosa, muscularis mucosae, submucosa, musculature, subserosa or perigastric structures for their bases, depending on their depth (Figs. 2, 3, 4, 6, 9, 11, 14, 19, 20, 29, 30, 34, 35, 37 and 38).

4. The excavations may be wide and shallow (Fig. 2) or narrow and deep (Figs. 8, 9, 37 and 38).

5. The mucosa of the borders of gastric ulcers may gradually recede from the base or overhang the base (Figs. 2, 14 and 34).

6. The epithelium of the mucosa, which is the most natural source of carcinoma, may be normal (differentiated), as in Figures 15, 16 and 24; hyperplastic (partially differentiated), as in Figures 23 and 25, or irregularly hyperplastic and migratory (extra glandular or in the stroma), as in Figures 31, 32 and 36.

7. The bases of chronic gastric ulcers consist of scar tissue which radiates usually from the center of the ulcer or is perpendicular to the plane of the coats of the stomach (Figs. 7, 12, 20, 30, and 35).

8. Usually the surface of the base is composed of necrotic tissue which is infiltrated with lymphocytes (Figs. 7, 12, 17, 20, 21, 30 and 35).

9. Carcinomatous cells occur in the lower parts of the mucosa and in the immediate submucosa without their being present in the base (Figs. 30, 31 and 32).

10. Of 684 specimens which were either excised or resected from the stomach, 191 were chronic ulcers or ulcers in which no histologic evidence of carcinoma was present. There were 472 specimens which presented the characteristics of simple ulcer plus the presence of carcinoma and twenty-one specimens of ulcer in which the presence of cancer was doubtful. The ulcer which contains the smallest amounts of carcinomatous cells contains these in the mucosa of the borders and not in the base. The earliest carcinomatous condition of the cells is not seen in so-called "cut-off" epithelium, in scar tissue of the base, but is seen in the epithelium of the mucosa (Figs. 26, 27, 31 and 32).

Without regard to clinical history, certain conclusions may be drawn from the facts enumerated above:

1. Single and multiple chronic ulcers occur in the stomach.

2. Single and multiple chronic gastric ulcers occur with all the characteristics of simple ulcers plus the presence of carcinomatous cells in their borders minus the presence of similar cells in the bases.

3. Simple and multiple gastric ulcers occur which present the macroscopic characteristics of simple ulcer plus the presence of carci-

noma in the borders and bases, indeed, with glandular involvement and metastases.

The question for the clinician and the surgeon is not "Does gastric carcinoma develop on gastric ulcers and what percentage of ulcers become malignant?" but "Has the proved association of gastric carcinoma with chronic gastric ulcer sufficient significance to be of prognostic value in cases clinically diagnosticated as gastric ulcer?"

The association of these two conditions in conjunction with the fact that the immediate operative mortality, the postoperative mortality and the subsequent duration of life after gastric resection bear a definite relation to the extent of the carcinomatous involvement[1] should be sufficient to stimulate a consideration on the part of every physician of the possibility of chronic gastric ulcers not only becoming but actually being carcinomatous.

The question of differential diagnosis in such cases may also be answered by facts, the most salient of which is the fact that the differential diagnosis is often to be made only by a cellular pathologist after the removal of the tissue and not by the clinician or surgeon.

Do we know of any method other than exploration and microscopic examination which positively answers the question of whether or not a specimen inside or outside the body is carcinoma? The clinical history, gastric analysis, roentgenoscopy and serum diagnosis cannot positively differentiate these groups of cases, especially those without pyloric obstruction. Indeed, the microscope, which is our present highest court of appeal, can do no more than to divide the specimens into three groups, namely, simple ulcer group, carcinoma group and a doubtful group. Whenever the clinician feels positive of the clinical diagnosis of chronic gastric ulcer he should consider that carcinoma can not be ruled by our present methods of clinical investigation.

1. MacCarty and Blackford: Involvement of Regional Lymphatic Glands in Carcinoma of the Stomach, Ann. Surg., June, 1912.

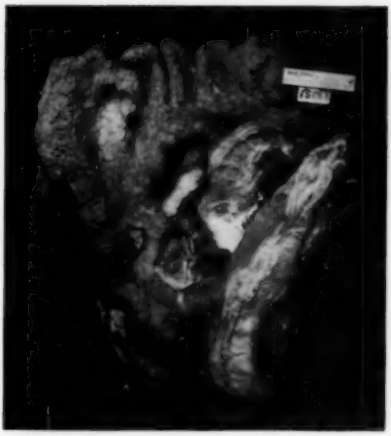


Fig. 1 (18088).—Multiple chronic gastric ulcers in various degrees of extension.

Fig. 2 (18088).—Gross section through four ulcers showing the degrees of extension through the wall of the stomach.

Fig. 3 (18088).—Low-power photograph through the smallest ulceration in Figures 1 and 2. The destruction has not extended through the muscularis mucosae. Some distortion of the glands and lymphocytic infiltration of the mucosa.

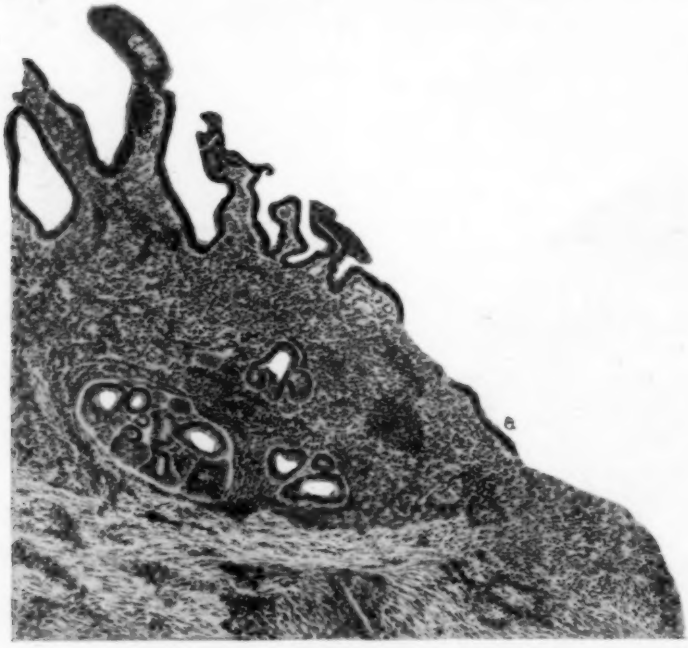


Fig. 4 (18088).—Low-power drawing through the wall and part of the base of the ulcer second in size in Figures 1 and 2. The destruction has extended through the muscularis mucosae and into the submucosa. There is extensive distortion of the glands and also lymphocytic infiltration.

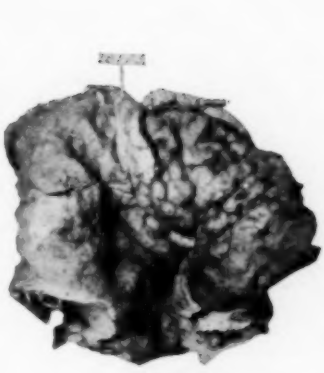


Fig. 5.

Fig. 6.

Fig. 5 (28173).—Single simple chronic gastric ulcer.

Fig. 6 (28173).—Gross section through the ulcer in Figure 5 showing the depth and extensive scar tissue of the base. The ulceration has extended through all of the gastric coats.

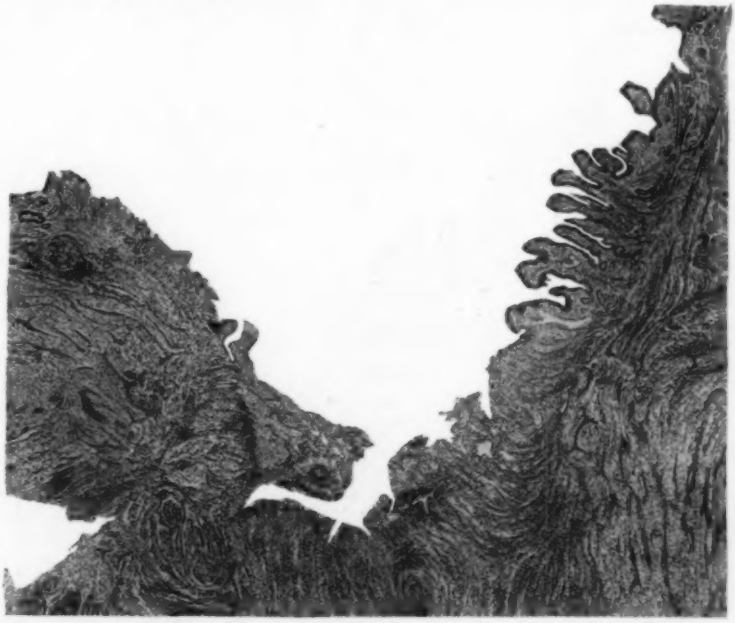


Fig. 7 (28173).—Low-power drawing through the specimen in Figures 5 and 6 showing the characteristics of a simple chronic gastric ulcer. Destruction, distortion of the mucosa, lymphocytic infiltration, necrosis of the tissue in the base, and the peculiar arrangement of the scar tissue in the base.

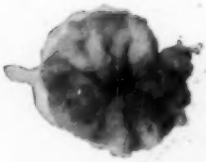


Fig. 8.

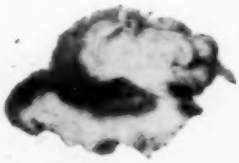


Fig. 9.

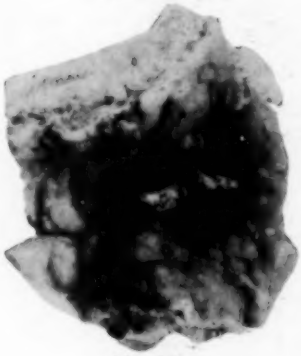


Fig. 10.

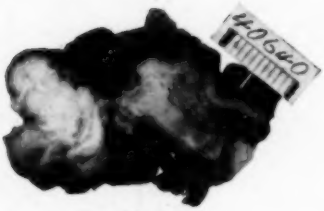


Fig. 11.

Figs. 8, 9, 10 and 11 (37378 and 40640).—Photographs showing simple chronic gastric ulcers.

Fig. 12 (27381).—Low-power drawing showing the characteristics of a simple chronic gastric ulcer.

Fig. 13.

Fig. 14.

Figs. 13 and 14 (49687).—Photographs of a simple chronic gastric ulcer.

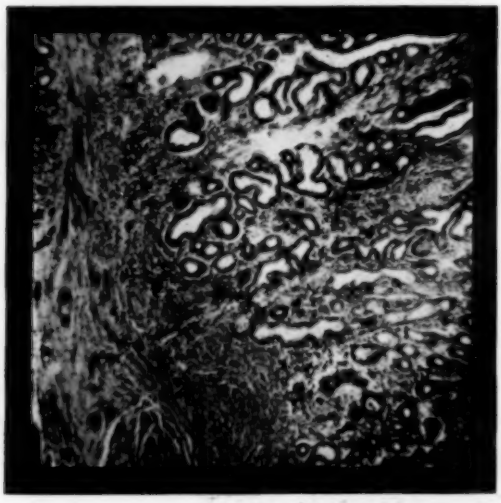


Fig. 15.

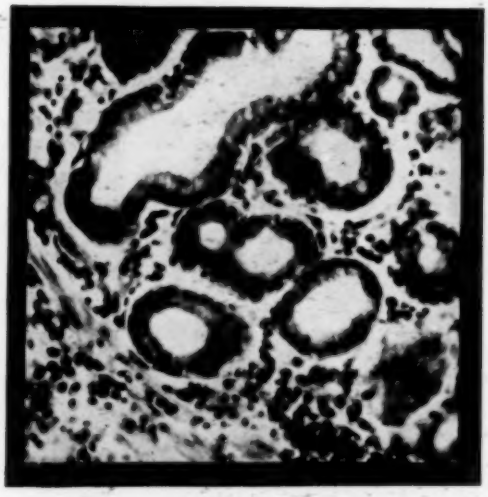


Fig. 16.

Figs. 15 and 16 (48401).—Low-power photographs showing the character of the glands of the mucosa in a simple chronic gastric ulcer. The epithelium consists of definitely differentiated cells which still retain the characteristics of the normal gastric epithelium.

Fig. 17 (48401).—Low-power photograph through the base of a simple chronic gastric ulcer showing lymphocytic infiltration and scar tissue.

Fig. 18.

Fig. 19.

Figs. 18 and 19 (A53435).—Photographs of a simple chronic gastric ulcer.

Fig. 20 (53630).—Low-power photograph through a simple chronic gastric ulcer.

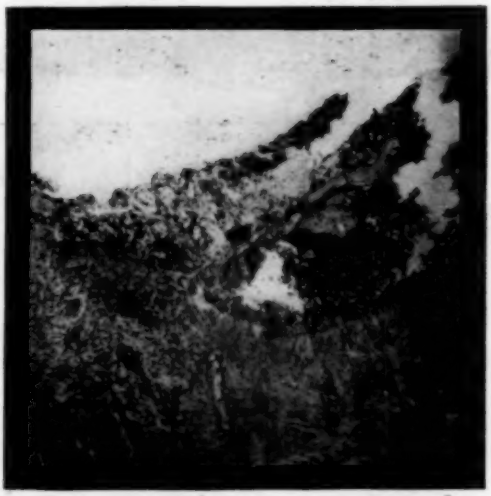


Fig. 21 (53630).—Low-power photograph through the base of the ulcer in Figure 21.

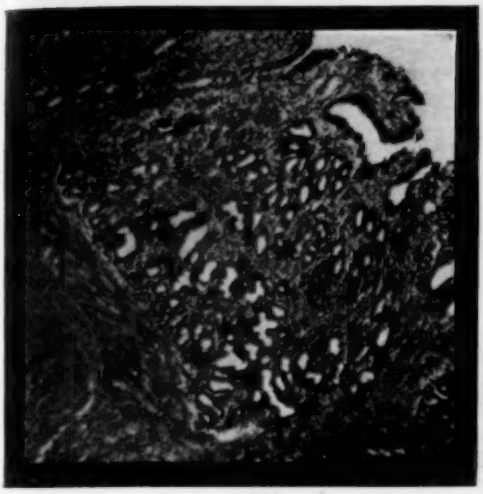


Fig. 22.

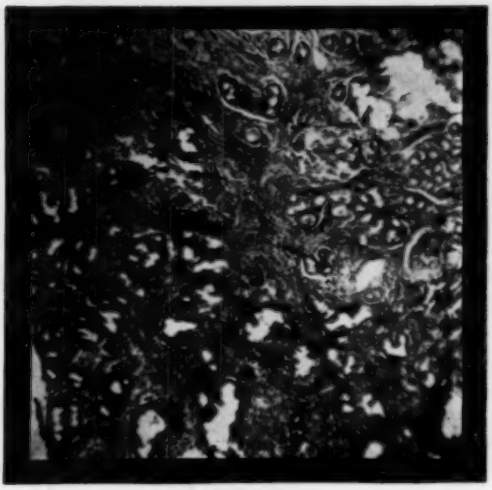


Fig. 23.

Fig. 24.

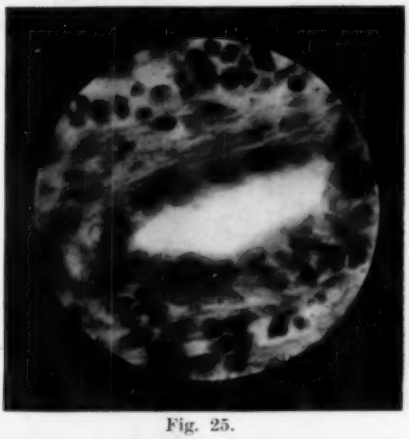


Fig. 25.

Fig. 26.

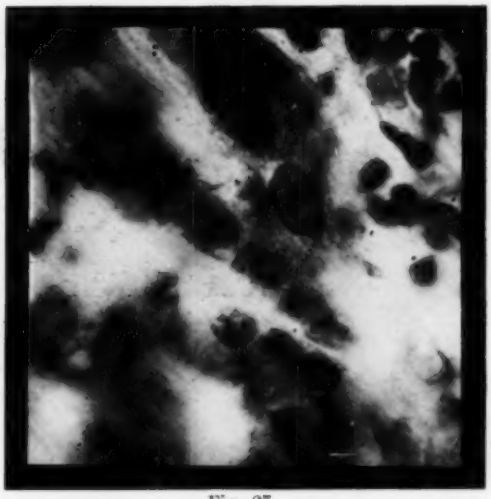


Fig. 27.

Figs. 22, 23, 24, 25, 26 and 27 (53630).—Photograph of the epithelium in the mucosa of the borders of a chronic gastric ulcer (Fig. 20). The glands are more irregular than those seen in Figures 15 and 16. The cells are not so typically differentiated as those in Figure 16, and still there is no definite invasion of the stroma. The cells resemble many of those which are seen in carcinoma (Figs. 31, 32 and 36).

Fig. 28.

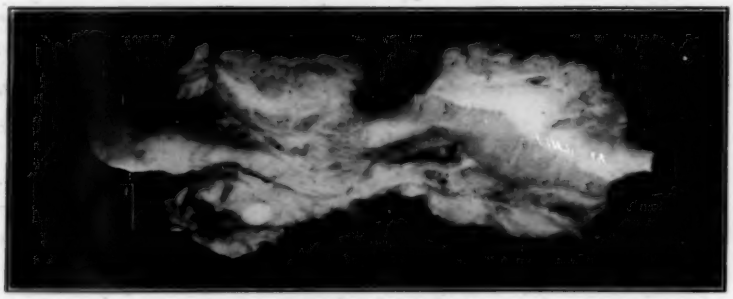


Fig. 29.

Figs. 28 and 29 (35572).—Photographs through a chronic gastric ulcer.

Fig. 30 (35572).—Low-power photograph through the ulcer which is seen in Figures 28 and 29. The characteristics of simple chronic gastric ulcer may be seen to exist. There is no apparent carcinoma in the base.

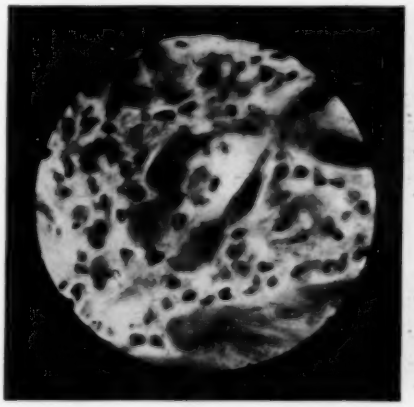


Fig. 31.

Fig. 32.

Figs. 31 and 32 (35572).—Photographs of undifferentiated, irregular epithelial cells in the submucosa just below the muscularis mucosae. The cells are typical of carcinoma.

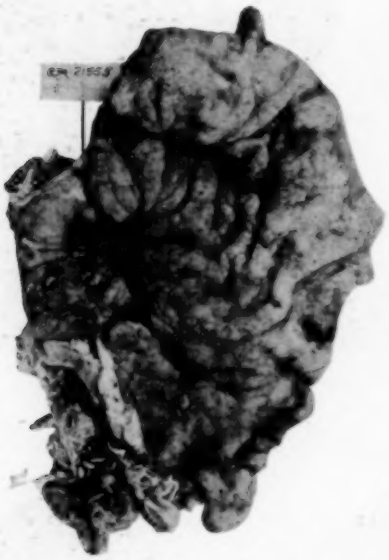


Fig. 33.

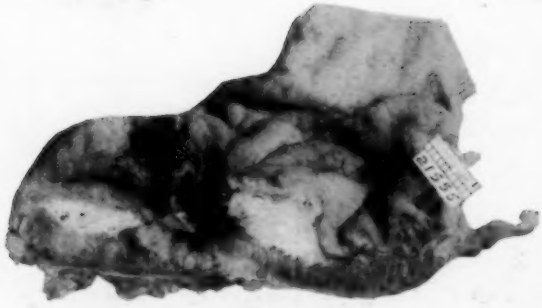


Fig. 34.

Figs. 33 and 34 (21555).—Photographs of chronic gastric ulcers.

Fig. 35 (21555).—Low-power drawing through the border and part of the base of the ulcer, showing the presence of carcinoma in the mucosa and just below the mucosa, but none in the base. Direct connection between the glands of the mucosa and the carcinoma may be seen.

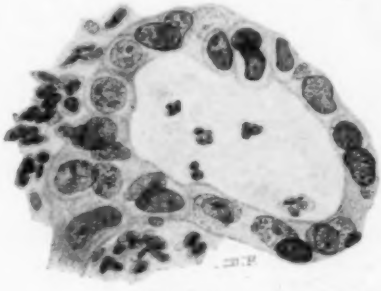


Fig. 36.

Fig. 37.

Fig. 36 (21555).—High-power drawing through an epithelial carcinomatous island in the submucosa of the gastric ulcer in Figs. 33 and 34.

Fig. 37 (21882).—Chronic gastric ulcer plus the presence of carcinoma.

Fig. 38 (21882).—Gross section through the carcinomatous ulcer which is seen in Figure 37.

THE NON-PROTEIN NITROGENOUS CONSTITUENTS OF THE BLOOD IN CHRONIC VASCULAR NEPHRITIS (ARTERIOSCLEROSIS) AS INFLUENCED BY THE LEVEL OF PROTEIN METABOLISM *

OTTO FOLIN, PH.D., W. DENIS, PH.D., AND MALCOLM SEYMOUR, M.D.
BOSTON

By virtue of the analytical technic recently introduced by Folin and Denis for the analysis of blood it has become possible to study the retention of nitrogenous waste products with far greater ease and certainty than formerly. Such studies are particularly to be desired in the investigation of nephritis, for urine analyses cannot reveal the stage of accumulation of such products, an accumulation which may vary between almost normal figures and those accompanying uremia. According to Strauss, the high blood-pressure of nephritis is usually or at least frequently accompanied by an excessive accumulation of the nitrogenous waste products, whereas edema is rather associated with a retention of chlorids. For the investigation reported in this paper we have selected a group of patients whose blood-pressure varied between 160 and 225 mm.

The chief purpose of our investigation was to determine the extent to which it is possible by means of diets to vary, particularly to reduce, the waste nitrogen in the blood of such nephritis and incidentally determine whether there is any relationship between the blood-pressure and the accumulations of nitrogenous waste products in the blood. With regard to the latter point, the details of our results will be described by one of us (Seymour) in a separate paper, though a few figures for the blood-pressure are recorded in Tables 1 to 12. We have included in the work a series of "kidney efficiency" tests, according to the phenolsulphonephthalein method recently introduced by Rowntree and Geraghty, in order to obtain further comparisons between the values given by that test and the actual accumulation as revealed by blood analyses. While hospital records were kept of the diet of each patient, we omit all such data and give the urinary nitrogen alone as the index of the total protein metabolism. The subjects of these experiments were inmates of an institution maintained on Long Island, Boston Harbor, by the city of Boston for the accommodation of indigent individuals. They were

* From the Laboratories of Biologic Chemistry and Theory and Practice of Harvard Medical School and the Long Island Hospital.

* Submitted for publication Oct. 6, 1913.

selected mainly on the basis of abnormally high blood-pressures, and were removed from the institute and placed in a special ward in the Long Island Hospital.

With the exception of Charles M., all of these patients were allowed to be out of bed during the day; but in order to control the collection of urine and the diets, they were not permitted to leave their own ward, except to go on an adjoining veranda. The patients were under the constant supervision of a special nurse and orderly, who were placed at our disposal for this purpose by the hospital authorities.

It was originally our intention to divide the time covered by the experiment into three periods: (1) a period in which the patients would be allowed to eat at will the ordinary ward diet; (2) a period of high protein feeding; (3) a period of low protein diet with high calorific intake. The diets proposed for the first and third periods were successfully administered, but during the period of high protein feeding we were, as will be seen on examination of the figures for urinary nitrogen, unable to raise the protein metabolism to a very high level. This failure was due to the nausea and vomiting which occurred in many cases during that part of the experiments.

During the second or "high protein" period beside the administration of a liberal supply of meat and eggs at the three regular meals, two to three egg-nogs daily were given between meals.

During the third or low protein period the diet consisted of apple-tapioca pudding, boiled or fried rice, boiled potatoes with butter, fruit (oranges, apples and stewed pears) and boiled cabbage. In addition, during this period, each patient took daily 150 to 200 gm. of arrowroot starch, which had been rendered soluble with diastase. This soluble starch was served hot, flavored with fruit juice or with a little port wine or whisky.

During the entire experiment smoking was allowed, and coffee was served three times daily.

The crucial point which we set out to determine, namely, whether by low nitrogen diets it is possible to reduce the urea and total non-protein nitrogen of blood to the normal or less is abundantly proven by the tabular records of the preceding twelve nephritic patients. In every case the figures for the retention nitrogen obtained at the end of the third period, the low protein period, are down substantially to the figures obtained from strictly normal persons (from 22 to 28 mg. non-protein nitrogen and from 11 to 14 mg. urea nitrogen).

The original level of retention was, to be sure, not very high in several of these patients. The patients had been selected chiefly on the basis of the blood-pressure, and high blood-pressure is not necessarily accompanied by excessive retention of nitrogenous waste products. Judging

TABLE 1.—Result of Test in Case 1.

Daniel B., aged 60, weight 63.5 kg. Diagnosis: Aortic roughening and regurgitation, chronic interstitial nephritis.

Diet	Date	Urine		Blood (100 gm.)			Phenol-sulphone-phthalein Excretion in 2 Hrs. Per Cent.	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	960	9.7	..	..	...	26	175
Ward	4/11/13	1,080	9.9					
Ward	4/12/13	930	9.7					
Ward	4/13/13	1,020	9.2					
Ward	4/14/13	1,050	8.4	40	18	1.9	22	145
High protein	4/16/13	1,020	8.9	..	..	...	..	160
High protein	4/17/13	1,080	10.3					
High protein	4/18/13	1,140	12.6	..	..	...	38	
High protein	4/20/13	1,560	11.2					
High protein	4/21/13	1,020	9.7	40	20	2.7	26	155
Low protein	4/23/13	1,020	7.9	..	..	...	..	140
Low protein	4/24/13	1,050	11.5					
Low protein	4/25/13	1,080	5.5					
Low protein	4/26/13	1,080	3.5					
Low protein	4/27/13	1,200	2.7	21	11	2.5	45	170
Average	.....	.....	....	..	..	...	31.6	

TABLE 2.—Result of Test in Case 2

Thos. H. M., aged 57, weight 80.5 kg. Diagnosis: Chronic interstitial nephritis, hypertrophy of heart, arteriosclerosis.

Diet	Date	Urine		Blood (100 gm.)			Phenol-sulphone-phthalein Excretion in 2 Hrs. Per Cent.	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	960	8.4	..	..	...	..	180
Ward	4/11/13	450	5.1					
Ward	4/12/13	780	7.4					
Ward	4/13/13	1,080	8.4					
Ward	4/14/13	1,140	8.0	44	26	1.7	12	190
High protein	4/16/13	900	7.5	..	..	...	..	185
High protein	4/17/13	1,020	8.1					
High protein	4/18/13	1,140	8.4	..	..	...	22	
High protein	4/20/13	1,620	12.4					
High protein	4/21/13	1,560	15.0	44	24	2.7	36	160
Low protein	4/23/13	1,440	9.8	..	..	...	..	190
Low protein	4/24/13	900	6.6					
Low protein	4/25/13	1,500	6.0					
Low protein	4/26/13	1,200	4.4					
Low protein	4/27/13	1,630	4.2	19	10	1.7	35	208
Average	.....	.....	....	..	..	...	26.2	

TABLE 3.—Result of Test in Case 3

William McG., aged 71, weight 67.5 kg. Diagnosis: Arteriosclerosis, chronic emphysema, chronic interstitial nephritis.

Diet	Date	Urine		Blood (100 gm.)			Phenolsulphonephthalein Excretion in 2 Hrs. Per Cent.	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	900	7.0	..	..	...	..	160
Ward	4/11/13	510	5.1					
Ward	4/12/13	900	9.0					
Ward	4/13/13	1,020	7.5					
Ward	4/14/13	1,350	7.8	38	20	2.2	34	160
High protein	4/16/13	1,020	9.2	..	..	...	..	160
High protein	4/17/13	905	8.2					
High protein	4/18/13	1,020	10.8	..	..	...	29	
High protein	4/20/13	1,380	11.3					
High protein	4/21/13	1,080	10.6	37	20	2.3	34	160
Low protein	4/23/13	1,920	7.4	..	..	...	..	145
Low protein	4/24/13	900	9.0					
Low protein	4/25/13	1,440	5.3					
Low protein	4/26/13	1,500	7.1					
Low protein	4/27/13	1,260	3.3	23	11	2.0	37	158
Average	.....	.....	....	..	..	...	33.5	

TABLE 4.—Result of Test in Case 4

Timothy, W. C., aged 71, weight 91.5 kg. Diagnosis: Right inguinal hernia, arteriosclerosis, senility, chronic interstitial nephritis.

Diet	Date	Urine		Blood (100 gm.)			Phenolsulphonephthalein Excretion in 2 Hrs. Per Cent.	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Nitrogen Protein mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	.....	....	..	..	...	..	175
Ward	4/11/13	570	5.4					
Ward	4/12/13	1,560	11.2					
Ward	4/13/13	1,230	8.8					
Ward	4/14/13	2,070	12.0	49	25	2.6	..	170
High protein	4/16/13	1,560	10.1	..	..	...	..	142
High protein	4/17/13	1,440	13.7					
High protein	4/18/13	1,560	14.8	..	..	...	31	
High protein	4/20/13	1,080	16.2					
High protein	4/21/13	1,440	18.7	48	23	2.6	39	165
Low protein	4/23/13	1,080	8.7	..	..	...	..	140
Low protein	4/24/13	1,050	10.4					
Low protein	4/25/13	1,620	6.5					
Low protein	4/26/13	1,980	5.9					
Low protein	4/27/13	2,040	5.2	22	10	1.8	50	158
Average	.....	.....	....	..	..	...	46.6	

TABLE 5.—Result of Test in Case 5

Nathan, D. L., aged 81, weight 78.5 kg. Diagnosis: Arteriosclerosis, senility, cardiac hypertrophy, chronic interstitial nephritis.

Diet	Date	Urine		Blood (100 gm.)			Phenolsulphonephthalein Excretion in 2 Hrs. Per Cent.	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	1,290	7.8	..	..	...	..	210
Ward	4/11/13	600	5.7					
Ward	4/12/13	1,110	7.8					
Ward	4/13/13	1,140	8.5					
Ward	4/14/13	1,590	12.2	50	28	2.7	14	198
High protein	4/16/13	1,260	8.4	..	..	...	..	188
High protein	4/17/13	990	8.5					
High protein	4/18/13	1,200	12.0	..	..	...	20	
High protein	4/20/13	1,440	11.0					
High protein	4/21/13	1,050	10.0	58	40	3.3	17	235
Low protein	4/23/13	1,440	6.7	..	..	...	..	170
Low protein	4/24/13	1,080	6.9					
Low protein	4/25/13	960	4.5					
Low protein	4/26/13	1,500	5.7					
Low protein	4/27/13	1,920	4.3	23	12	2.0	28	203
Average	.....	.....	....	..	...	...	19.8	

TABLE 6.—Result of Test in Case 6

Martin, T. D., aged 54, weight 71.5 kg. Diagnosis: Arteriosclerosis, chronic interstitial nephritis.

Diet	Date	Urine		Blood (100 gm.)			Phenolsulphonephthalein Excretion in 2 Hrs. Per Cent.	Pressure Blood-
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	1,260	11.1	..	..	...	35	235
Ward	4/11/13	1,050	10.5					
Ward	4/12/13	930	6.4					
Ward	4/13/13	1,530	7.8					
Ward	4/14/13	1,710	8.7	39	18	3.0	33	187
High protein	4/16/13	1,020	8.3	..	..	...	..	200
High protein	4/17/13	1,500	13.1					
High protein	4/18/13	1,260	9.6	..	..	...	23	
High protein	4/20/13	2,040	14.1					
High protein	4/21/13	1,620	13.6	62	45	3.9	30	180
Low protein	4/23/13	1,320	8.5	..	..	...	..	160
Low protein	4/24/13	1,200	7.7					
Low protein	4/25/13	1,500	6.1					
Low protein	4/26/13	1,560	5.6					
Low protein	4/27/13	1,560	4.8	30	15	2.7	29	190
Average	.....	.....	....	..	..	...	30	

TABLE 7.—Result of Test in Case 7

Edward R., aged 74, weight 59.5 kg. Diagnosis: Inguinal hernia, arteriosclerosis, chronic interstitial nephritis.

Diet	Date	Urine		Blood (100 gm.)			Phenol-sulphone-phthalein Excretion in 2 Hrs. Per Cent.	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	1,260	7.4	..	..	...	..	225
Ward	4/11/13	840	6.8					
Ward	4/12/13	1,410	9.4					
Ward	4/13/13	1,350	10.3					
Ward	4/14/13	1,250	9.8	50	28	2.2	21	190
High protein	4/16/13	1,380	9.1	..	..	...	..	200
High protein	4/17/13	1,140	9.5					
High protein	4/18/13	1,860	11.5	..	..	...	29	
High protein	4/20/13	1,920	11.7					
High protein	4/21/13	1,380	11.1	82	67	3.9	26	180
Low protein	4/23/13	1,500	6.7	..	..	...	..	190
Low protein	4/24/13	1,440	7.5					
Low protein	4/25/13	1,560	4.9					
Low protein	4/26/13	1,080	3.3					
Low protein	4/27/13	900	1.9	26	12	2.3	35	190
Average	.....	.....	....	..	..	...	28	

TABLE 8.—Result of Test in Case 8

Michael O'C., aged 66, weight 90.5 kg. Diagnosis: Arteriosclerosis, chronic emphysema, cardiac hypertrophy, chronic interstitial nephritis.

Diet	Date	Urine		Blood (100 gm.)			Phenol-sulphone-phthalein Excretion in 2 Hrs. Per Cent.	Pressure Blood-
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	1,110	10.1	..	..	...	..	195
Ward	4/11/13	720	6.7					
Ward	4/12/13	1,050	11.4					
Ward	4/13/13	1,260	9.9					
Ward	4/14/13	1,170	11.1	28	14	2.5	34	198
High protein	4/16/13	1,440	11.1	..	..	...	..	240
High protein	4/17/13	1,380	11.3					
High protein	4/18/13	1,020	10.6	..	..	...	23	
High protein	4/20/13	1,446	14.4					
High protein	4/21/13	1,680	15.3	30	15	2.5	..	210
Low protein	4/23/13	1,080	10.8	..	..	...	..	180
Low protein	4/24/13	1,320	10.9					
Low protein	4/25/13	1,440	6.3					
Low protein	4/26/13	1,020	2.4					
Low protein	4/27/13	1,500	3.8	20	11	2.6	30	150
Average	.....	.....	....	..	..	...	29	

TABLE 9.—Result of Test in Case 9

William T. W., aged 59, weight 70.5 kg. Diagnosis: Left hemiplegia, mitral regurgitation and aortic roughening, chronic interstitial nephritis, arteriosclerosis.

Diet	Date	Urine		Blood (100 gm.)			Phenolsulphonephthalein Excretion in 2 Hrs. Per Cent.	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	720	4.8	..	..	...	..	175
Ward	4/11/13	480	5.1					
Ward	4/12/13	1,050	5.4					
Ward	4/13/13	1,020	9.8					
Ward	4/14/13	1,110	7.4	27	14	2.6	..	170
High protein	4/16/13	1,020	7.0	..	..	...	..	172
High protein	4/17/13	1,080	9.6					
High protein	4/18/13	1,080	9.0	..	..	...	26	
High protein	4/20/13	1,560	13.0					
High protein	4/21/13	.....	....	30	16	2.8	31	140
Low protein	4/23/13	1,500	7.0	..	..	...	..	160
Low protein	4/24/13	1,050	7.8					
Low protein	4/25/13	1,200	3.5					
Low protein	4/26/13	720	2.6					
Low protein	4/27/13	1,620	3.3	20	11	2.3	24	190
Average	.....	.....	....	..	..	...	27	

TABLE 10.—Result of Test in Case 10

John De W., aged 82, weight 80 kg. Diagnosis: Cardiac hypertrophy, arteriosclerosis, chronic interstitial nephritis.

Diet	Date	Urine		Blood (100 gm.)			Phenolsulphonephthalein Excretion in 2 Hrs. Per Cent.	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	750	7.3	..	..	...	23	170
Ward	4/11/13	540	5.8					
Ward	4/12/13	600	6.4					
Ward	4/13/13	1,110	9.2					
Ward	4/14/13	1,470	7.9	30	15	2.5	35	150
High protein	4/16/13	1,020	8.2	..	..	...	..	154
High protein	4/17/13	1,200	12.0					
High protein	4/18/13	960	12.5	..	..	...	16	
High protein	4/20/13	2,040	10.1					
High protein	4/21/13	1,500	19.6	30	15	3.0	18	145
Low protein	4/23/13	1,020	7.7	..	..	...	..	135
Low protein	4/24/13	1,050	9.8					
Low protein	4/25/13	1,440	5.3					
Low protein	4/26/13	1,560	4.7					
Low protein	4/27/13	1,560	3.4	19	10	...	20	140
Average	.....	.....	....	..	..	...	28	

TABLE 11.—Result of Test in Case 11

Frank M., aged 72, weight 89.5 kg. Diagnosis: Arteriosclerosis, cardiac hypertrophy, chronic interstitial nephritis.

Diet	Date	Urine		Blood (100 gm.)			Phenol-sulphone-phthalein Excretion in 2 Hrs. Per Cent.	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	1,110	15.0	..	..	...	..	225
Ward	4/11/13	570	5.6					
Ward	4/12/13	1,050	8.5					
Ward	4/13/13	1,560	9.5					
Ward	4/14/13	2,040	9.6	30	15	2.6	29	190
High protein	4/16/13	1,440	11.5	..	..	...	..	220
High protein	4/17/13	1,560	12.3					
High protein	4/18/13	1,560	14.2	..	..	...	27	
High protein	4/20/13	1,560	13.0					
High protein	4/21/13	1,920	14.8	32	16	2.8	39	200
Low protein	4/23/13	1,020	7.0	..	..	...	..	180
Low protein	4/24/13	1,440	7.5					
Low protein	4/25/13	1,680	4.8					
Low protein	4/26/13	1,620	4.6					
Low protein	4/27/13	2,100	4.2	19	10	2.0	33	200
Average	.....	.....	....	..	..	...	32	

TABLE 12.—Result of Test in Case 12

Chas. McL., aged 63, weight 90.5 kg. Diagnosis: Cardiac hypertrophy, arteriosclerosis, chronic interstitial nephritis.

Diet	Date	Urine		Blood (100 gm.)			Phenol-sulphone-phthalein Excretion in 2 Hrs. Per Cent.	Pressure Blood-
		c.c. Vol.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	1,740	13.9	..	..	...	..	150
Ward	4/11/13	960	10.5					
Ward	4/12/13	1,020	11.1					
Ward	4/13/13	1,200	9.3					
Ward	4/14/13	1,530	9.5	30	14	3.0	13	160
High protein	4/16/13	1,440	9.9	..	..	...	..	139
High protein	4/17/13	1,560	12.2					
High protein	4/18/13	1,500	15.5	..	..	...	45	
High protein	4/20/13	1,920	18.3	37	20	2.9	33	140
Low protein	4/23/13	2,400	12.5					
Low protein	4/24/13	1,380	8.4					
Low protein	4/25/13	2,400	6.2					
Low protein	4/26/13	2,400	4.6					
Low protein	4/27/13	1,560	3.4	20	11	2.1	28	140
Average	.....	.....	....	..	..	...	29.7	

from our figures, there clearly is no marked connection between the blood-pressure and the degree of retention; indeed, it is doubtful whether there is any connection. A high blood-pressure, therefore, does not necessarily indicate the desirability of introducing low nitrogen diets in nephritis. So far as can be seen at the present time, the only criterion available for regulating the protein content of the food in nephritis is the determination of the amount of retention by blood analysis. Five of the twelve patients (the last five) used in our experiments showed little or no abnormal retention of waste nitrogen, and there is no reason to doubt that these could take care of the protein contained in the usual hospital diet.

So far as the phenolsulphonephthalein test for the kidney efficiency is concerned, it is to be noted that there is no correspondence between the results of this test and the degree of waste nitrogen retention. Since it is possible to increase or reduce the retention by protein feeding one cannot expect any close agreement between it and the phenolsulphonephthalein test. The lack of correspondence is, however, probably not to be explained entirely on the basis of different levels of total protein metabolism.

In Table 13 we give the nitrogen elimination, the non-protein nitrogen and the phenolsulphonephthalein excretion — all in terms of the average for the first two periods of the experiments.

TABLE 13.—AVERAGE IN CASES 1 TO 12 OF URINARY NITROGEN, NON-PROTEIN NITROGEN AND PHENOLSULPHONEPHTHALEIN EXCRETION*

Case	Urine Nitrogen gm.	Blood Non-Protein Nitrogen, mg.	Phenolsulphonephthalein Excretion Per Cent.
9	8.0	28.5	27.0
3	8.7	37.5	33.5
2	8.9	44.0	26.2
5	9.2	54.0	19.8
7	9.7	61.0	28.0
1	9.8	40.0	31.6
6	10.3	50.5	30.0
10	10.9	30.0	28.0
8	11.0	29.0	29.0
11	11.4	31.0	29.5
12	12.3	33.5	29.7
4	14.3	48.5	46.6

* Average for first two periods arranged according to urinary nitrogen.

If 60 to 80 per cent. be taken as the normal elimination of 6 mg. of phenolsulphonephthalein excretion when injected intramuscularly it is clear that this elimination can be reduced by one-half or more before there need be any abnormal accumulation of waste nitrogen in the blood.

TABLE 14.—Result of Blood Tests in Cases 13 to 18

Case	Name	Age	Diagnosis	Blood									Phenol-sulphone-phthalein Excretion Per Cent. in 2 Hrs.
				Ward Diet			High Protein Diet			Low Protein Diet			
				Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.	
13	*Patrick M.	63	Chr. interstitial neph.	92	70	3.5	124	109	4.5	..	..	...	8
14	Mary K. O.	75	Chr. interstitial neph.	42	34	2.5	61	41	3.5	..	..	...	54
15	Hannah J..	64	Chr. interstitial neph., aortic roughening, aneurysm of arch.	50	34	2.1	58	40	2.5	36	16	2.4	32
16	Mary K....	81	Chr. interstitial neph., cardiac hypertrophy, aortic roughening, mitral regurgitation	35	19	1.9	39	25	1.9	30	16	2.0	30
17	†Chas. M...	60	Chr. interstitial neph., epilepsy ...	34	19	2.6	69	55	2.0	23	11	...	25
18	Dennis O'B.	31	Chr. interstitial neph., albuminuric retinitis, alcoholic ...	58	41	2.0	‡75 80	52 62	2.1 2.5	44	20	2.1	6

* This patient died in "uremia" while on high protein diet two days after last sample of blood was taken.

† This patient was confined to bed during almost the whole period of experiment. Urinary nitrogen determinations made March 4 to April 4 showed nitrogen excretion varied from 22 to 15 gm. in twen ty-four hours. Went on low protein diet, as given in first twelve cases, April 23. During a five-day period nitrogen excretion average in twenty-four hours was 5 gm.

‡ Taken one week apart.

It would seem from these results as though the direct determination of the non-protein nitrogen (and urea) in the blood furnishes a more reliable guide to what might be called the protein tolerance of patients than can be obtained from any "direct" test of kidney efficiency, for of all tests yet devised for this purpose the phenolsulphonephthalein test of Rowntree and Geraghty is admittedly the best.

In addition to the preceding experiments we have made a number of others which are less complete, but from which some noteworthy observations were obtained. These experiments were made before the series shown in Tables 1 to 13, and while in every case collections of urine were made it was suspected that in a number of instances the whole twenty-four-hour amount had not been saved. We, therefore, have not included our determinations of urinary nitrogen, but have been forced to rely on the dietary records kept by the nurses in charge.

As in the preceding experiments, eggs and meat were used in liberal amounts during the periods of high protein feeding. In the low protein periods, however, the patients were fed on cereals, bread and butter, vegetables and a little milk. The soluble starch employed so extensively in our later experiments was not used, so that in some cases it is to be feared that the calorific intake was too low.

UROBILIN: ITS CLINICAL SIGNIFICANCE*

RAY LYMAN WILBUR, M.D., AND THOMAS ADDIS, M.D.
SAN FRANCISCO

I. INTRODUCTION

The numerous studies made on urobilin and urobilinogen during the forty-four years since its discovery by Jaffé, while adding much to our knowledge of its chemistry and its clinical occurrence, still leave much to be desired as to methods for its estimation and as to its clinical significance. Some investigators are inclined to look on it still from the point of view of hemoglobin metabolism, while others consider it only in reference to hepatic insufficiency. Its relationship to the presence or absence of bile in the intestine has been a subject of considerable dispute, but in the main the contention of Friedrich von Müller, that it can originate only from the bacterial decomposition of bile reaching the intestine, is accepted. Nevertheless, the assertion of Fischler, that it can originate in the liver itself, seems to us probable. Whether it is then formed within the liver cell or is produced by decomposition of bilirubin in the biliary passages, it is impossible to state. A wide variability in point of view is reflected throughout the available literature. It has been our intention in entering on a study of this substance to endeavor to estimate its present value as an aid in diagnosis and prognosis, and we must frankly admit that it has been disappointing in some particulars, owing largely, perhaps, to the imperfections of the present methods of its estimation, and to its evident instability. Besides, when such variable factors as the bacterial fermentations within the intestine, the absorption from it, the activity of the liver, the elimination of bile, the eliminative power of the kidney, the rate of destruction of the red blood-corpuscles, the activity of the spleen, etc., have to be taken into account, it is not surprising that difficulty should arise in interpreting findings and in correlating the diverse views of many investigators and clinicians.

The interesting spectroscopic fluorescent and color tests for urobilin and its mother substance urobilinogen and their common occurrence in the body excretions, especially in disease, naturally stimulated much research as to their composition and origin and many clinical observations as to their significance. The Ehrlich aldehyd test, shown by

* Submitted for publication July 1, 1913.

* From the Laboratory of Experimental Medicine, Leland Stanford Junior University, Department of Medicine.

Neubauer to be due to urobilinogen, received particular attention. The method of estimation with Citron's or other simple spectroscope of the urobilin and urobilinogen together proved with us to be fairly simple, and to lend itself readily to approximate quantitative studies based on the disappearance of the characteristic absorption bands between D and E, and B and F, with successive dilutions.

The term "urobilin" is not applied to a single body, but to a group of derivatives of blood and bile pigments containing the pyrrol nucleus. Throughout our work we have considered urobilin and its mother substance urobilinogen as practically the same, and have endeavored always to estimate them together in making clinical and experimental observations. The confusion between the two and the lack of a generally accepted theory of the origin of these substances is the cause of the present striking lack of uniformity in the interpretation of clinical and laboratory results.

The most prominent of the various theories as to the origin of urobilin are the hematogenous, the hepatogenous, the nephrogenous, the histogenic and the enterogenous. The latter is undoubtedly the most satisfactory, although it does not fully explain the origin of all urobilin within the body, for instance, that of some hepatic disorders (Fischler) and some cases of blood destruction and extravasation. In brief, these theories are as follows:

Hematogenous Theory.—This theory is that the urobilin can be, but is not necessarily, derived directly from blood pigment. It is based on the somewhat uncertain evidence of the long-known occurrence of urobilinuria in hemorrhage of the brain (von Bergmann), in blood extravasations (Kunkel), in extra-uterine pregnancy (Dick), in hemorrhagic ascites (such as the famous case of Gerhardt, in which there was also a carcinomatous closure of the ductus choledochus), after hemocytolytic processes, in sulphonal poisoning, in paroxysmal hemoglobinuria and in scurvy. While it is true that there is the possibility in many of these reported clinical observations of the existence of definite hepatic disturbance, still there is enough experimental evidence to warrant the assertion that at times urobilin can be derived directly from blood pigment without the intervention of either the liver or the intestine.

Hepatogenous Theory.—The principal tenet of this unsatisfactory theory is that there is failure on the part of the liver in its normal decomposition of hemoglobin, and that as a result of this insufficiency urobilin is formed and reaches the kidney through the blood-stream and is there eliminated, or is added to the intestinal contents by the bile. A somewhat strict interpretation of this theory is that there is a direct formation of urobilin within the liver cell, or at least within the bile passages from the bile pigment.

Nephrogenous Theory.—In brief, this is based on the fact that urobilin as such is very rarely found in the blood, and that it reaches the kidney in the form of bilirubin, which is reduced to urobilinogen in its passage through the renal epithelium. Herscher states that kidney substance can reduce bilirubin, but surviving kidneys with bilirubin circulating in their blood secrete no urobilinogen, but only bilirubin in the collected urine. The principal argument against this theory is that urobilin does not occur in many cases of jaundice.

Histogenic Theory.—This theory that hemoglobin or bile pigments are reduced in the tissues to urobilin and then reabsorbed and eliminated, offers an insufficient explanation of the various known phenomena.

Enterogenous Theory (Friedrich von Müller).—While not meeting all conditions, this theory is based on the explanation of the established facts of urobilin formation. In brief, it is based on the finding that bile pigment reaching the intestinal lumen undergoes a change into urobilinogen and urobilin caused by bacterial decomposition. It may then be absorbed into the capillaries of the portal system and so reach the liver parenchyma. If the liver is normal the major portion of the urobilin is broken up or synthesized to bile or blood pigment. A portion may be secreted in the bile and so be returned to the intestinal lumen to be reabsorbed or passed off with the stool. When the liver parenchyma is damaged, these changes in the urobilin may not take place, but it passes on into the general circulation. It reaches the kidney through the blood and is eliminated in the urine. This theory is principally sustained by the absence of urobilin in the urine in any quantity in cases of obstruction of the common duct.

We shall not stop for a complete discussion of these theories, since points of view vary, and there is no common agreement at present. We can safely assume that as a rule the presence of urobilinuria is indicative of the fact that bile is reaching the intestine, and that large amounts of urobilin in the urine usually mean that there has been either abnormal destruction of red blood-corpuscles, or an insufficiency of the liver to perform its normal functions. A large amount of urobilin in the stool indicates increased blood destruction. Urobilinuria frequently disappears in severe diarrhea. Although urobilin is normally present in the urine in small quantities, its presence cannot be ascertained by the ordinary qualitative tests — a positive reaction with Schlesinger's zinc acetate test or Ehrlich's aldehyd test is not found in normal urine. It is increased in many pathological conditions. It is commonly increased in infectious diseases, in diseases in which there is wide-spread hemorrhage, traumatic or otherwise, in pernicious anemia, malaria, cardiac decompensation, various forms of liver and gall-passage disease, and in numerous intoxications such as those due to alcohol, chloroform, lead and carbon monoxid.

II. SUMMARY OF QUANTITATIVE METHODS FOR UROBILIN ESTIMATION

Following is a summary of only the most essential points of the methods which have been used:

Almost all the work done has been with the urine. The methods may be divided roughly into those requiring a separation of the urobilin from the urine by means of precipitation with ammonium sulphate; secondly, those depending on the spectroscopic absorption bands of urobilin, and thirdly, those based on the property of fluorescence and lastly colorimetric methods depending on the violet color produced by copper sulphate in urobilin solutions.

1. Methods Involving Salting out of Urobilin by Means of Ammonium Sulphate.—Hoppe-Seyler in 1891 estimated urobilin in urine gravimetrically. The acidified urine was saturated with ammonium sulphate, and the precipitate extracted with chloroform and alcohol. The solution was evaporated, the residue dissolved in ether, filtered and evaporated, and the residue dissolved in alcohol, evaporated and weighed.

Viglezio in the same year recommended a method which depended on the salting out of the urobilin with ammonium sulphate and its solution in alcohol. The amount was gaged by noting how much of this solution was required to produce a certain grade of fluorescence or the appearance of the spectroscopic absorption bands, when it was added to a solution of zinc chlorid.

Studenski added copper sulphate to the urine, precipitated with ammonium sulphate and dissolved the copper compound of urobilin in chloroform. This color was compared with that produced by copper sulphate in a solution of urobilin of known strength.

Ladage recognized the necessity of taking the urobilinogen into account and recommended that iodin should be added to convert it to urobilin. The urobilin was then precipitated by saturation of the urine with ammonium sulphate, and the acid chloroform solution diluted until the spectroscopic absorption became invisible. In Fr. Müller and Huppert's method a mixture of barium chlorid and barium hydrate is added to the urine, and the precipitate is filtered off and washed with hot water. The excess of barium in the filtrate is removed by sodium sulphate, and the filtrate after neutralization with sulphuric acid is saturated with ammonium sulphate. The precipitated urobilin is filtered off, allowed to dry in the air and extracted three times after acidification with a warm mixture of alcohol and ether. This solution is then evaporated to a convenient bulk and the amount of urobilin estimated spectrophotometrically.

Charnas in 1909 gave us an alternative method to one based on the spectroscopic characteristics of urobilin, a gravimetric method in which after all urobilinogen in an ether extract of the urine has been converted into urobilin by exposure to sunlight, the urobilin is separated by water from the ether, and after filtration precipitated by ammonium sulphate, dried, dissolved in alcohol, the alcohol evaporated *in vacuo* and the residue weighed.

2. Spectroscopic Methods.—Gerhardt (1889) and Beck (1895) made estimations by means of the spectrophotometer using Vierordt's tables of the grade of light extinction in different regions of the spectroscope in normal urine and solutions of urobilin.

Saillet (1897) extracted the freshly passed urine with acetic ether. In another specimen the urobilinogen was converted into urobilin by exposure to light and then extracted. The two extracts are added together and diluted until the spectroscopic band disappears.

Conner and Roper (1908) made approximate measurements of the amount of urobilin in urine by noting the number of dilutions required to obliterate the urobilin band in the urine. They recommend the addition of a few drops of Lugol's solution to the urine in order to convert the urobilinogen into urobilin.

Auche (1909) found that a solution of urobilin of 1 : 200,000 prepared by a method he describes is sufficient to make the five absorption bands of potassium permanganate all of equal intensity, although without it some are lighter than others. He therefore superimposes the urine, which in some cases has to be extracted with a thymol-chloroform solution, over a solution of potassium permanganate, and dilutes the urine or its extract until all the bands are of equal intensity.

Simpson (1910) acidifies the urine with sulphuric acid and exposes it to light for some time in order to convert urobilinogen to urobilin. It is then diluted until the urobilin band disappears. This method gave fairly accurate results when a dilution of fifteen or more volumes was necessary. With smaller amounts the reading was apt to be obscured by other pigments.

Hausmann (1913) adds copper sulphate to the urine and dilutes until the spectrum becomes invisible.

Henocque, Hayem, Gautretet, Deniqués, Hildebrandt, Riva and Zoja have used one modification or another of these spectroscopic methods.

Charnas (1909) makes the urine alkaline, allows it to ferment at 37 C. (98.6 F.) for from twenty-four to forty-eight hours, acidifies with tartaric acid, and extracts with ether and petroleum benzin. To a portion of the ether extract an ethereal solution of paradimethylamidobenzaldehyd is added and two to three drops of absolute alcohol saturated with hydrochloric acid gas. After dilution if necessary with alcohol a spectrophotometric reading is made.

3. Methods Depending on the Property of Fluorescence.—Grimm (1893), Fischler (1906), Grigant and Monod (1909), and Descomps (1909) gage the amount of urobilin by the intensity of the fluorescence present in the zinc salt filtrate of the urine. Various devices are adopted to aid the conversion of urobilinogen into urobilin.

4. Colorimetric Methods.—Bogomaloff (1892) used a colorimetric method depending on the depth of the red-violet color produced in a chloroform extract of urine containing urobilin by the addition of copper sulphate.

Braunstein (1903) used a modification of this method.

Flatow and Brünell (1913) estimate the amount of urobilinogen in fresh urine from the depth of the red color given on the addition of Ehrlich's reagent. As a standard they use a phenolphthalein solution to which a few particles of metallic sodium have been added.

Brugsch and Retzlaff (1912) modify Charnas' method by extracting the alkaline urine first with ligroin. They make the final reading with Plesch's colorimeter, using a solution of Bordeaux red as the standard.

The Stools

Quantitative estimations of urobilin in the stools have been carried out by comparatively few investigators. Gerhardt at first used a somewhat complicated process of extraction and purification but later except in special cases simply extracted with acid alcohol and read spectrophotometrically. Auche extracts with alcohol, removes other pigments by shaking with ligroin and thereafter makes the same spectroscopic determination as he employs in urine extracts.

Simpson (1910) mixes the day's feces with water and adds enough dilute sulphuric acid to make the mixture distinctly acid. For one or two days full exposure to light is allowed to convert the chromogen into urobilin. Filtration is then carried out and the residue repeatedly extracted until the filtrate becomes colorless. Direct examination of the filtrates is made and the amount determined from the degree of dilution required to obliterate the spectroscopic band.

Brugsch and Retzlaff (1912) attempt to convert all the urobilin into urobilinogen by allowing the stools to stand for some time before extracting with ligroin to remove indol derivatives and then with acetic ether. After the addition of paradimethylamidobenzaldehyd they make a colorimetric determination as in their method with urine.

Serum

No quantitative work has been done with serum. The methods employed for the detection of urobilin in serum were reviewed by Conner and Roper. We shall refer therefore only to those which have appeared since their publication.

Morel and Monod (1908) add alcohol to the serum and heat to boiling for half an hour. The filtrate is concentrated and a drop of Obermeyer's reagent and an alcoholic solution of zinc acetate and acetic acid are added. After twenty-four hours the filtrate is examined for fluorescence.

Auche (1909) adds a solution of iodin in potassium iodid to the serum and afterward some zinc cyanate in ammonia and filters. The filtrate is examined spectroscopically.

Fromholdt and Nersessoff (1912) precipitate the oxalated plasma with alcohol, filter, concentrate, acidify, and shake with amyl alcohol, and then with alkaline water. The water is then made acid and shaken with amyl alcohol and examined spectroscopically.

III. DISCUSSION OF METHODS

In reviewing the literature on these methods, it was evident that the one main difficulty encountered by all investigators was the problem of separating urobilin and urobilinogen from the other coloring matters present in the excretions without at the same time destroying more or less of it. Many had frankly given up the attempt to attain any but the roughest purification, and had relied on the intensity of the physical properties of fluorescence or of light absorption. In doing so they had, of course, confessed that their results were not quantitatively accurate, for variations in the impurities present would undoubtedly affect the basis on which their determinations rested. Others have not remained satisfied with this, but have attempted to attain at any rate approximately pure solutions before estimation.

The question as to which of these two methods produced the better result depended on whether more was gained in accuracy than was lost by destruction of urobilin in the various precipitations, separations and evaporations required.

It appeared to us that there was one way in which the question could be answered. When undiluted urine is examined spectroscopically, nothing is seen, as a rule, except a general absorption of light, which becomes deeper and deeper toward the blue end of the spectrum. Even if urobilin is present, the absorption band cannot be clearly differentiated. When the amount of urobilin, however, is much larger than the other light-absorbing substances present, one finds that on simple dilution with water the band between E and F becomes more and more distinct, so that it at last stands out sharply. On still further dilution it begins to fade, and a more or less definite point is reached at which it becomes invisible when the full amount of light is allowed to pass through the spectroscope, but can still just be seen when the light is diminished. In some urines one may have to carry the dilution to forty or more times the original volume of urine to reach this point. At this dilution the disturbing effect of other substances is negligible. Such urines afford an

excellent means of deciding as to whether any particular manifestation leads to a loss of urobilin or not, because the number of dilutions required to remove the urobilin band before and after the procedure can be very readily determined. We were fortunate at this time in having under observation for several months a patient with bronzed diabetes in whose urine unusually large amounts of urobilinogen and urobilin were constantly present.

Practical experience with this method very quickly showed us that any and every tried means which may be applied to separate urobilin from the urine was associated with more or less loss. Extracts from precipitations with ammonium sulphate, separations by ether, chloroform or thymol-chloroform solutions, and processes of evaporation, with or without heat, all led to a greater or less diminution of the spectroscopic dilution value. Such methods are applicable in the endeavor to obtain pure urobilin, but it seems to us that they can have no place in any quantitative estimation, for the amount destroyed is too variable and too incapable of control. The one salient fact which has to be kept constantly in mind in choosing a method of estimation is that almost any manipulation is apt to be associated with more or less loss of urobilin. It is better to recognize from the first that ordinary chemical methods are not applicable to a substance which is so labile, and that their employment gives only a fallacious appearance of accuracy. Any gravimetric method for this reason seems to us to be entirely out of question. Solutions of urobilin or urobilinogen may no doubt be obtained in a state of sufficient purity to admit of spectrophotometric determination, but with the means which have hitherto been employed for this purpose the error by loss so far outweighs any increase of accuracy in the final determination as to make results obtained by such methods of very doubtful value.

Yet in the case of all urines in which the amount of urobilin was not exceptionally large and in dealing with stools, some preparation was necessary, for no direct reading could be made. After trying a large number of procedures, the conclusion was reached that the most innocuous of all was to mix equal parts of urine and a saturated solution of zinc acetate in alcohol and to filter. This is Schlesinger's qualitative test for urobilin. A small amount of urobilin may be absorbed by the precipitate, and at first we filtered under pressure and washed the precipitate with alcohol; but latterly we have found that the error is so small compared with other unavoidable sources of error that in routine clinical examinations we have simply taken the first fraction of the filtrate.

Another necessary procedure also soon became apparent. It has, of course, long been known that on adding acid to a urobilin solution the position of the absorption band is altered, but we had not gathered that the intensity was changed to the extent which we found in many urines.

Sometimes the increase in the number of dilutions required to obliterate the band was ten times as great in filtrates which had been made strongly acid as in the fresh filtrate. It was obvious that unless constancy could be attained in this point, no results of value could be obtained. The intensity of the spectroscopic characteristics of urobilin are as dependent on reaction as is the property of fluorescence. The difference is that to attain maximum fluorescence a nice balancing of reaction is required, whereas the strongest absorption band is obtained in a strongly acid solution and an excess of acid does no harm. All that is required, therefore, is to be sure that enough acid is present. For this reason alone the spectroscopic methods are to be preferred to those based on fluorescence in which large errors may arise from slight variations in reaction.

Still another essential point in any method is that the urobilinogen as well as the urobilin shall be taken into account, and that if possible the quantitative relationship between the two bodies shall be established so that the result can be expressed as a single figure. Urobilin is simply an oxidation product of urobilinogen; from the clinical or physiological points of view they have exactly the same significance. Urobilinogen is potential urobilin. In any specimen of urine or stool the relative amounts of the two are constantly changing, the amount of urobilin increasing and of urobilinogen decreasing the longer the exposure to light and air. In some methods this difficulty is met by keeping the extract for twenty-four hours exposed to light, and it is assumed that all the urobilinogen will then have been converted into urobilin. Others endeavor to hasten the process by the addition of oxidizing agents, and one method takes the opposite course, and by alkaline fermentation, attempts to convert all the urobilin into urobilinogen.

The only way by which urobilinogen can be determined, except gravimetrically, is by the spectroscope through the absorption bands of the pigment produced by its reaction with paradimethylamidobenzaldehyd. We used a solution containing 20 gm. in 300 c.c. of 50 per cent. concentrated hydrochloric acid. One c.c. was added to every 10 c.c. of the alcoholic zinc acetate filtrate. This gave a sufficient amount of acid to produce the maximum intensity in the urobilin band and at the same time brought out the urobilin spectrum, so that the dilution values of both urobilin and urobilinogen could be read in the same filtrate. The amount of urobilinogen could then be expressed as representing so many dilutions in the same way as with the urobilin.

The first point investigated was the effect of light on urobilinogen. It was found to vary greatly with the intensity of the light. A few hours' exposure to direct sunlight suffices to cause the complete disappearance of urobilinogen. With indirect light the process required a longer time; but after exposure to a diffuse light in a room with ground-glass win-

dows, there was found after twenty-four hours to be an almost complete disappearance of urobilinogen. The figures in Table 1 show the effect of leaving the zinc acetate filtrates of urine in diffuse room-light for twenty-four hours.

TABLE 1.—Effect of Leaving Zinc Acetate Filtrates of Urine in Diffuse Room-Light for Twenty-Four Hours

Urobilinogen

Filtrate Examined at Once	Examined After 24 Hours in Room-Light
32	1
37	0
45	2
25	0
21	1
12	0
7	0
16	0
34	0
40	1
18	1
25	0
19	0
40	0

The effect on urobilin of a similar exposure to light could not be determined in the same way, since all fresh urine and stools contain urobilinogen which passes over in part at least to urobilin. It was invariably found, however, in these specimens in which by various means the urobilinogen had been removed, that light had a destructive effect on urobilin, as was shown by the diminution in the dilution value after exposure.

Under the influence of light, as the urobilinogen diminishes the urobilin increases. The question remained, however, as to how much loss of urobilinogen and urobilin occurred during this process. The precise degree of loss could be found only if we knew the quantitative relationship between urobilinogen and urobilin and the relative light-absorbing powers of the urobilinogen and urobilin pigments. These facts are not known, but an indication of the amount of loss of urobilin was gained by comparing the total dilution value obtained by adding together the urobilinogen and urobilin values in the fresh filtrates, in those which had been in darkness for twenty-four hours and in those which had been left in diffuse room-light for the same time.

After twenty-four hours in darkness there had been a diminution of urobilinogen from 371 to 301 and an increase of urobilin from 6 to 134. The light-absorbing power of urobilin must then either be greater than that of the urobilinogen pigment, or one molecule of urobilinogen must break up into two or more molecules of urobilin. In any case there is an increase of the total dilution values for urobilinogen and urobilin

together, associated with a diminution of urobilinogen and an increase in urobilin.

TABLE 2.—Quantitative Change in Urobilinogen and Urobilin Under the Influence of Light

Fresh Urine		After 24 Hours in Dark		After 24 Hours in Room-Light	
Urobilinogen	Urobilin	Urobilinogen	Urobilin	Urobilinogen	Urobilin
32	2	28	11	1	26
37	0	30	12	0	32
45	0	40	12	2	37
25	0	22	8	0	17
21	0	19	14	1	16
12	2	10	7	0	3
7	2	6	3	0	5
16	0	14	7	0	15
34	0	30	12	0	21
40	0	35	13	1	30
18	0	14	10	1	16
25	0	14	9	0	16
19	0	7	4	0	8
40	0	32	12	0	24
371	6*	301	134†	6	266‡

* Total "Urobilin" 371 + 6 = 377.
† Total "Urobilin" 301 + 134 = 435.
‡ Total "Urobilin" 6 + 266 = 272.

In the filtrates exposed to light there is more than forty times as much urobilin as in the fresh filtrates and twice as much as in those kept in the dark, and yet in spite of that the total dilution value is much less, only 266 as compared with 377 and 435. If for a moment it is assumed that the diminution of urobilinogen and increase in urobilin in the filtrates kept in the dark represent the relationship between the two bodies, then 371 — 301 = 70 of urobilinogen should produce 134 — 6 = 128 of urobilin. But the disappearance of 371 — 6 = 365 of urobilinogen in the filtrates exposed to light led to an increase of only 266 — 6 = 260 of urobilin instead of the 667 which should have resulted. It is impossible to explain this decrease in total dilution value except as due to a destruction of part of the urobilinogen or urobilin or both. Since the actual amount of urobilinogen or urobilin destroyed by exposure to light cannot be calculated, and varies so greatly with different degrees of intensity of light and with other unknown conditions, the conclusion was reached that it was not a means which could be used in any quantitative method to convert urobilinogen into urobilin, and that as far as possible exposure to light should be avoided in urobilin estimations.

Attention was then turned to the effect of oxidizing agents which have been recommended especially by French investigators; but here

also the results were disappointing, for those reagents which were effective in oxidizing the urobilinogen were also very destructive to the urobilin.

Charnas states that if urine is made alkaline and left for from twenty-four to forty-eight hours in the incubator, all the urobilin will be converted into urobilinogen, which can then be estimated by the spectrophotometer. It is noteworthy that Charnas does not give any estimations indicating the decrease of urobilin and increase of urobilinogen during the process, nor does he bring forward any evidence to show whether or not any part of the urobilinogen or urobilin is destroyed by the fermentation which occurs. It has long been known that in freshly passed urine urobilinogen is present alone or at any rate in far greater amount than urobilin, suggesting that as it is excreted through the kidneys it is all in the form of urobilinogen; and it has been the universal experience that the longer a urine is kept the less urobilinogen will be found in it, so that the statement that in alkaline urine left exposed to the air at a temperature of 37 C. the reverse process occurs, and that instead of an oxidation a reduction takes place, is not one which can at once be accepted. We know that in the intestine there is a reduction of urobilin to urobilinogen, but there conditions are present much more favorable to reduction processes than those which are present in urine left exposed to air. Outside the body the formation of urobilin constantly goes on; but to bring about the reverse process the strongest reducing agents we know have to be employed. It seemed to us, however, that the question was one which could be decided by the simple spectroscopic method we have outlined.

Readings of the relative amounts of urobilin and urobilinogen in thirty specimens of urine before and after alkaline fermentation at 37 C. were made. In most of the cases fermentation was allowed to continue for twenty-four hours. The first twelve results are given in Table 3.

TABLE 3.—UROBILIN AND UROBILINOGEN BEFORE AND AFTER ALKALINE FERMENTATION AT 37 C.

Before Fermentation		After Fermentation	
Urobilinogen	Urobilin	Urobilinogen	Urobilin
15	5	0	11
12	0	0	9
26	9	7	0
15	5	0	0
12	0	6	0
7	0	8	3
26	9	0	2
19	3	5	2
0	3	0	11
0	5	30	0
17	6	0	1
10	5	50	2

Of these thirty specimens there was a diminution instead of an increase of urobilinogen after fermentation in all except three. In two there was a very considerable increase of urobilinogen, but both these specimens contained some bile, and as we found that urobilinogen was produced in decomposing bile we are inclined to ascribe these exceptions to this cause.

It seemed that these results, so completely at variance with the statement of Charnas as to the effect of alkaline decomposition in urine, might be due to the urobilinogen having become absorbed in the heavy deposit of phosphates in the fermented urines. The experiment was therefore repeated in ten urines, the readings before and after fermentation being made after the specimns which had been made faintly alkaline with ammonium carbonate had again been made acid with tartaric acid. Before fermentation a total reading of urobilinogen 18 and urobilin 27 was made. After twenty-four hours' fermentation, urobilinogen 13 and urobilin 16 was recorded, and after forty-eight hours, urobilinogen 12 and urobilin 4. We must conclude then that alkaline fermentation in the dark at a temperature of 37 C. not only does not quantitatively convert urobilin into urobilinogen, but that it has a directly destructive action on both.

Since there was no available way of converting all the urobilinogen into urobilin or vice versa, the only alternative was to make estimations of each separately. But could such results be added together and expressed by a single figure? Our knowledge of the chemistry of these bodies is not yet sufficiently advanced to enable us to be sure that one or the other may not be a condensation of the other so that one molecule of urobilinogen, for instance, may break up into two or more molecules of urobilin. No idea as to the relationship between them could be gained by noting the degree of increase in the dilution value of urobilin which followed a decrease in urobilinogen, for the factors concerned are too complex and variable to allow of any true idea being obtained in this way. For while the urobilinogen is being converted into urobilin, both are undergoing other changes and are losing the spectroscopic characteristics on which our determinations rest. In preparations of urobilin and urobilinogen made from bilirubin by Fromholdt's method we found such a lack of relation between the weight of bilirubin and the weight of urobilin produced, and such a variability in the relative amounts of urobilin and urobilinogen formed, that it did not seem likely that in this way either could we successfully determine whether or not urobilin and urobilinogen were equivalent in value. In dealing with two such extremely unstable substances whose presence can be detected only by a single physical property and which are constantly changing into other bodies which we have no means of estimating, the problem of their

exact relationship is one whose solution will probably not be definitely established until we have more knowledge of the decomposition products of hemoglobin. For urobilinogen and urobilin are only links in the chain of hemoglobin derivatives; they cannot be regarded as final end-products. The problem which we were attempting to solve was not the exact quantitative determination of urobilin, however, but simply the question as to whether or not any information of clinical value could be gained from urobilin estimations, in spite of the errors necessarily involved in any of the present methods. With this end in view the best that could be done was to add together the dilution values of urobilinogen and urobilin. We do not know that they are equivalent, but, on the other hand, we know that they are not very different in value. The proportion of the two substances varies so much in different specimens of urine, stool and bile that no comparison can easily be made if the dilution values for each are given separately.

As a result of this preliminary review we determined that the best method would be one which avoided all but absolutely essential manipulation of the material, one which was based on the most characteristic of the properties of urobilin, the spectroscopic absorption bands, and one in which the attempt to convert all the urobilinogen into urobilin or vice versa was abandoned and the results of separate estimations of the two were simply added together. Throughout this paper when the term "urobilin" is used apart from "urobilinogen," the sum of the dilution values of urobilin and urobilinogen is intended.

The following are the details of the methods which we finally adopted:

IV. METHODS

Urine

The method of collection of the twenty-four-hour urine has a considerable effect on the total spectroscopic reading. The vessel in which the urine is collected should be of dark brown glass and should be kept in darkness. Thymol crystals should be added, for even in cases in which no obvious fermentation had occurred we sometimes found a diminution in the total amount if no preservative was present. After measuring the amount of the twenty-four-hour urine, 10 c.c. are mixed with 10 c.c. of a saturated alcoholic solution of zinc acetate, and after a few minutes filtered. If a number of urines are being examined at the same time it is convenient to have test-tubes graduated to 10 and 20 c.c. Ten c.c. of the filtrate are taken and 1 c.c. of Ehrlich's solution[1] is added. It was found that this amount produced a sufficient concentration of acid in the mixture to give the maximum intensity of the urobilin band and contained enough of the paradimethylamidobenzaldehyd for the reaction with urobilinogen. The development of the urobilinogen band is not instantaneous. We found that as a rule it had attained its full intensity in a quarter of an hour. The action can be greatly accelerated by heating, but this is to be avoided. It is better to wait for an hour before making the reading, and during this time the solution should be kept in

1. Paradimethylamidobenzaldehyd, 20 gm.; concentrated hydrochloric acid, 150 c.c.; water, 150 c.c.

the dark. After three or four hours there is a diminution of the urobilin and urobilinogen in filtrates from urines, so the estimation should be made not later than three hours after adding the Ehrlich solution. We found that Citron's hand spectroscope[2] was the most convenient instrument to use. The filtrate was washed into a graduate and diluted with tap-water until first one and then the other bands of light absorption had disappeared when the full amount of light entered the spectroscope, but were still visible when the light was partly shut off. This gives a fairly definite end-point, and we did not find any great variation in the readings made by different persons. It is important of course that the light shall be always of approximately equal intensity. We made the readings in a dark room with a Tungsten electric bulb, holding the spectroscope close to the source of light. In highly colored urines one may be in doubt as to whether or not a trace of urobilin is present, for there may be so much general absorption of light as to obscure the urobilin band in the undiluted filtrate. There is no such difficulty with the urobilinogen band which lies between the red and yellow where there is no marked light absorption. With urines containing bile, if the amount of urobilin is not very large, it is necessary to add some fullers' earth and to leave the mixture standing for some time before filtration. If this is done the urobilin band can usually be read even in the undiluted filtrate. The dilution required gives the value for 5 c.c. of urine. If this figure is multiplied by the number of 5 c.c. quantities in the twenty-four-hour urine, the number of dilutions which would have been necessary if all the urobilinogen and urobilin in the twenty-four-hour amount had been concentrated in a volume of 5 c.c. is obtained. For instance, if in a twenty-four-hour urine measuring 1,000 c.c. a reading of ten dilutions for urobilinogen and of twenty for urobilin were made, the total urobilin would be $30 \times 200 = 6{,}000$. We tried to determine the dilution value of definite weights of urobilin prepared from bilirubin, but different preparations varied so much in their dilution value that it was obvious that we were not dealing with pure urobilin and we abandoned any attempt to express our results in milligrams of urobilin.

Stools

All the feces passed in the twenty-four hours were collected in the same receiver, the stools being protected from light. They were then washed into a large graduate and thoroughly ground up with water into a homogeneous paste, and water added to 0.5, 1, or if necessary 2 liters, depending on the quantity of stool. After thorough mixing, 25 c.c. were taken and 75 c.c. of acid alcohol (95 per cent. alcohol, 1,600 c.c.; concentrated hydrochloric acid, 25 c.c., and water, 800 c.c.) were added. The mixture was then put into a shaker for about half an hour. A considerable number of extractives were tried for removing the urobilin from the stools, but none were found so efficient as alcohol with hydrochloric acid. After thorough mixing in the shaker an equal quantity of a saturated solution of zinc acetate in alcohol was added and the mixture was filtered. After adding 2 c.c. of Ehrlich's reagent to 20 c.c. of the filtrate, the solution was put aside in a dark place until next day. The addition of zinc acetate is not absolutely essential, but in some cases we observed an intensification of the urobilin band following its use, and it is perhaps an advantage to make the urine and stool readings as far as possible under the same conditions. The development of the urobilinogen band was not always complete until six hours had elapsed, but thereafter there was no loss of urobilinogen or urobilin for a long time, although this was not the case with the zinc acetate filtrates before the addition of Ehrlich's solution. The reading was made in the same way as with the urine and the total amount calculated for the volume of stool after grinding up with water, the dilution of the 25 c.c. by acid alcohol and zinc acetate being taken into account. Instead of using tap-water for dilution of the final extract, 60 per cent. alcohol was required to avoid the development of a precipitate.

2. This can be obtained from Paul Altmann, Luisenstrasse, Berlin, Germany.

Bile

A measured amount of bile was mixed to a paste in a mortar with fullers' earth and left for some hours. An equal amount of the alcoholic solution of zinc acetate was then added and the mixture filtered under pressure, and the remainder washed with measured amounts of zinc acetate alcohol. In this way almost all of the bilirubin was absorbed, and though no doubt there was some loss of urobilinogen and urobilin, the comparatively small amounts found in the washings compared with the quantities present in the first filtrate seemed to indicate that the fullers' earth had very much less capacity for absorbing urobilin than bilirubin. Small amounts of urobilin or urobilinogen could no doubt be overlooked by this method. The Ehrlich solution was added in the same proportion as in the urine, but the dilutions had to be made with alcohol instead of water.

Blood-Serum

We have tried various methods, but so far we have not been successful in demonstrating urobilin with certainty.

V. THE EXCRETION OF UROBILIN IN URINE

Urobilin is a normal constituent of the urine, but the amount is so small under ordinary conditions that it can be demonstrated only by extracting large quantities of urine.

With the methods we have used, a positive result in twenty-four-hour specimens of urine indicates an abnormal increase over the amount usually present. We have only very rarely been able to find a faint and doubtful degree of absorption in the region of the urobilinogen or urobilin bands in the urines of persons in apparent health. When, therefore, urobilin is spoken of as being absent from urine, it is understood that an absence of an increased amount of urobilin is meant.

Saillet found in two normal persons that the quantity of urobilin excreted during the eight hours of sleep was only about one-tenth of the total twenty-four-hour amount. He gives two curves of the excretion during the daytime which show some relation to the body-temperature variations. He suggests a connection with the processes of digestion and of general metabolism.

In a case of bronzed diabetes with a large urinary excretion of urobilin, we had an opportunity to study the excretion from hour to hour. The patient was on a von Noorden strict diet plus 50 gm. of bread. His habits were very regular. He rose at 7 a. m. and went to sleep at about 9 p. m. Urine was passed every two hours during the daytime, and the amount of urobilin determined at once. The amount passed during the six or eight hours of sleep was kept in the dark and the urobilin determined at the earliest opportunity. In such freshly passed specimens, practically all the urobilin is in the form of urobilinogen, and the probable error arising from the assumption that the urobilinogen and urobilin readings represent equivalent values is avoided. For over a month, the two-hourly excretion was determined every day, with the result that there

was invariably found to be a marked diminution in the amount excreted at night. A graphic representation of the excretion during three consecutive days is given in Chart 1. The chart is typical of the results throughout the whole period. It shows the decrease of urobilin in the night urine and the great variation in the quantity from day to day and from hour to hour. The only constant feature is the fall during the night. Otherwise there is complete irregularity and the hour of maximum elimination occurred at any time during the day.

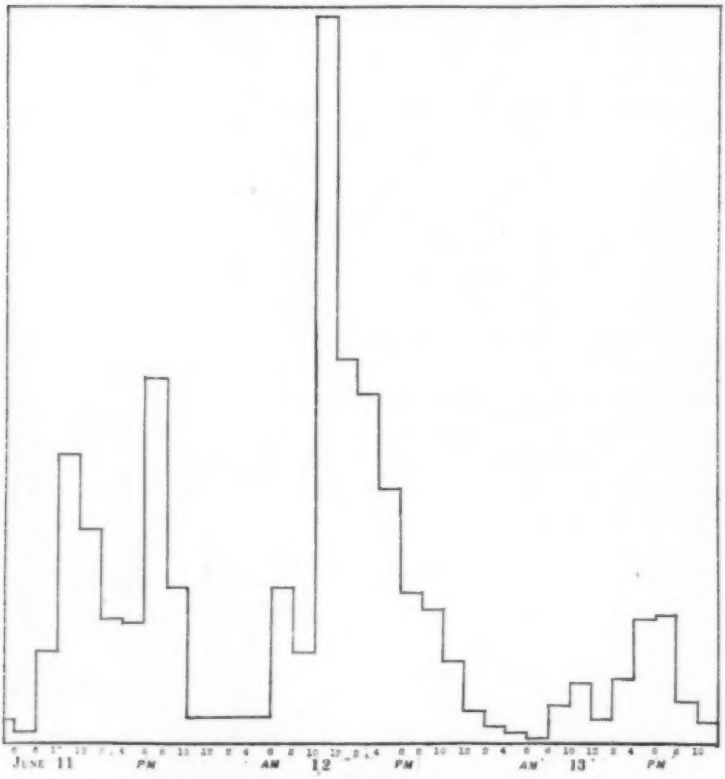


Chart 1.—Excretion of urobilin, June 11, 12 and 13.

When the patient remained up and took his meals during the night and slept in the daytime, the amount excreted in the night was much larger than in the day.

Charts 3, 4, 5, 6 and 7 show the relation between the excretion of water, total solids, sugar, chlorids and urea and urobilin. The relation was only a very general one, for the urobilin excretion shows irregular and wide fluctuations.

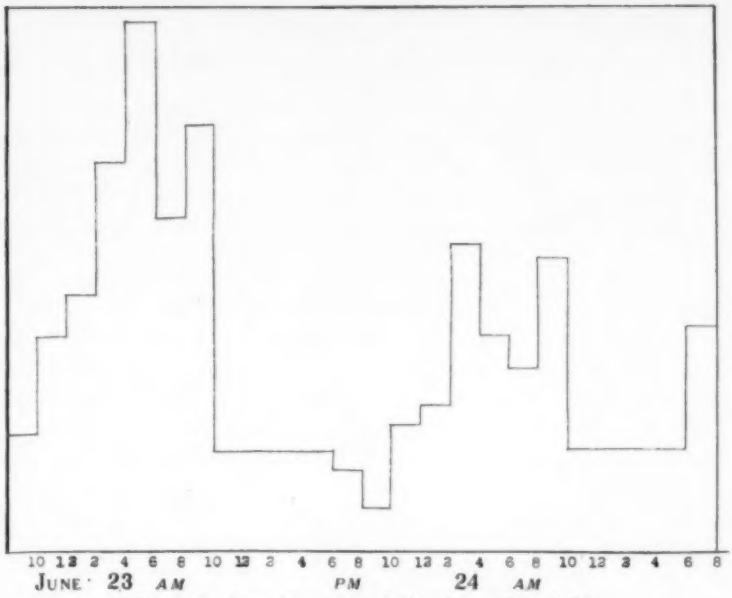


Chart 2.—Excretion of urobilin, June 23 and 24.

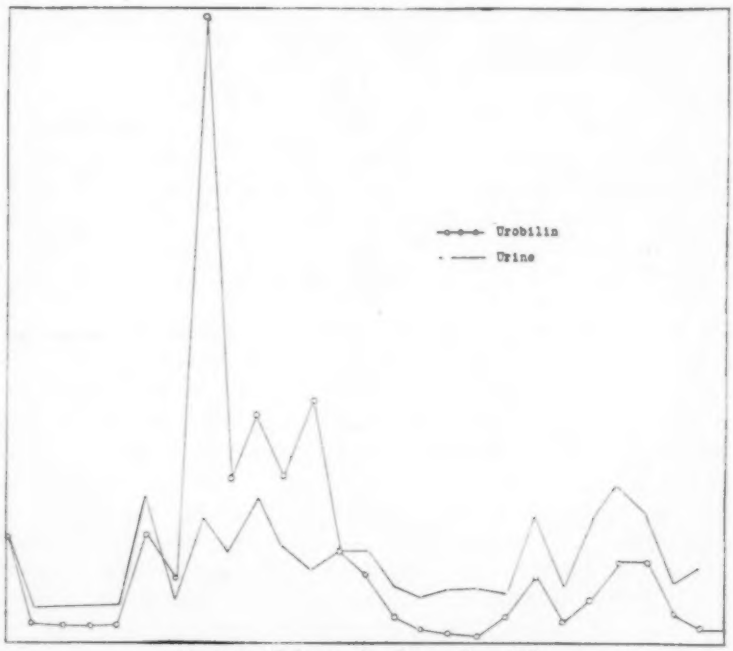


Chart 3.—Volume of urine and urobilin.

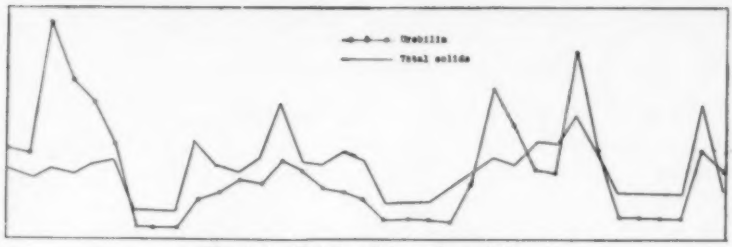


Chart 4.—Total solids and urobilin.

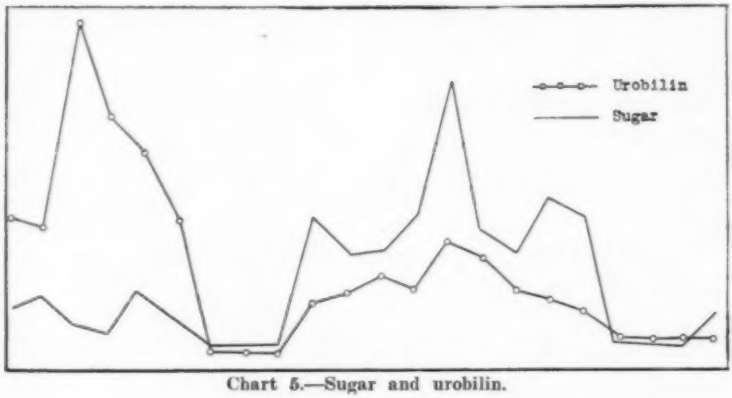


Chart 5.—Sugar and urobilin.

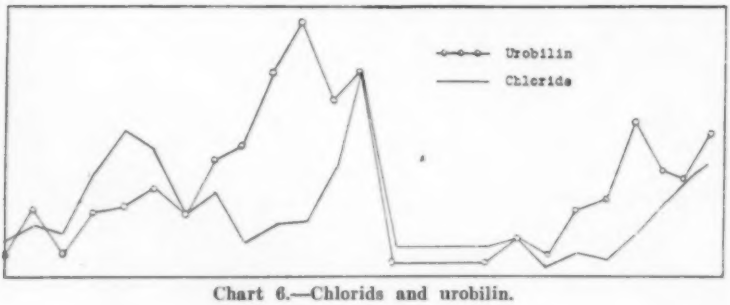


Chart 6.—Chlorids and urobilin.

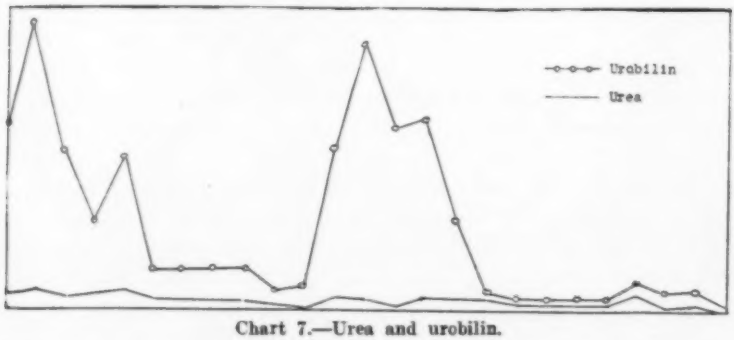


Chart 7.—Urea and urobilin.

These results appear to us to be of interest in connection with the question of urobilin excretion by the kidneys. It is generally agreed that most of the urobilin is formed in the intestine and is carried by the portal vein to the liver. There it is supposed to be either broken down further or resynthesized to bilirubin or hemoglobin (Brugsch) except for a very small part which is carried into the general circulation and is excreted through the kidneys. When the liver is diseased, the destruction or rebuilding of urobilin fails more or less, so that a greater quantity of the urobilin absorbed from the intestine passes by the liver to the kidneys.

The wide fluctuations in the quantities of urobilin in this case can scarcely be due to variations in the amount of absorption from the intestine. It is true that bile enters the duodenum only at intervals, but they are regular intervals when the periods of digestion are regular, and on these charts no signs of any periodicity during the daytime can be traced. In any case, the bile passes through the small intestine in about four hours, and as our findings as to the amount of urobilin in various parts of the intestinal tract show, the greatest quantity of urobilin is in the large intestine, not in the small. Urobilin is always present in the large intestine, and from this situation one could not expect any considerable changes from hour to hour in the amount absorbed. Urobilin is a readily diffusible substance, and since water is removed from the large intestine, it seems probable that urobilin will also be taken up by the blood.

Nor does it seem that changes in the excretory functions of the kidneys can account for more than a small part of the urobilin variations. The amounts of sugar and chlorids fall during the night mainly because their absorption from the alimentary tract almost ceases and not because there is any marked decrease in the eliminating functions of the kidneys. But urobilin is present in the large intestine in as large amount during the night as during the day, and if absorption is uniform one would expect uniform excretion, or at least no greater variation than might be ascribed to alterations in the circulatory and nervous mechanism of the kidneys. We have no evidence of any specific diminution in excretory power of the kidneys for any substance during the night.

If the absorption and excretion of urobilin appear to be too continuous and uniform to explain these charts, can we regard the variations shown in them as indicative of fluctuations in the power of the liver to keep the urobilin reaching it from the intestine from entering the general circulation?

The patient had an extremely large liver which post mortem was found to be cirrhotic and to contain a large amount of the pigment found in cases of hemochromatosis. During the day, when the work of the liver was increased because of the absorption of food products, it might

be imagined that the liver would be less able to cope with the urobilin brought to it, and the great increase during the daytime might be thus explained. But the wide and irregular fluctuations from day to day and from hour to hour, in spite of the fact that the meals were taken at regular intervals, remain to be accounted for. Possibly some other factor such as a formation of urobilin in the diseased liver may have played a part. In Saillet's two curves of hourly urobilin excretion in people in health, no such wide variations are to be seen.

Other possible factors which may play a part in alterations of the urinary excretion of urobilin are the changes which urobilin undergoes in the blood and tissues. The form in which urobilin circulates in the blood-stream is still unknown, and the question as to whether it may not there in part be broken up or synthesized to some other substance, or may not be stored in some form or other in the tissues, is still unsolved, and it is possible that in them an explanation may be found for these anomalies of urobilin excretion. At present, they can only be recorded.

VI. THE EXCRETION OF UROBILIN IN THE STOOLS

In ten adults in whom there was no reason to suspect any disturbance of hemoglobin metabolism, the average daily excretion in the stools was worked out. These cases, as shown in Table 4, showed variations between 3,307 and 8,737, and a general average of 6,475.

TABLE 4.—AVERAGE DAILY EXCRETION OF UROBILIN IN STOOLS OF TEN ADULTS WITH NORMAL HEMOGLOBIN METABOLISM

Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin in Stools
8	Inguinal hernia	8,685
15	Fractured femur	3,855
3	Chronic appendicitis	6,080
3	Endometritis	3,307
6	Neurasthenia	4,887
10	Neuritis	8,702
15	Chronic arthritis	6,604
11	Neurasthenia	8,737
6	Fractured femur	5,840
6	Fractured tibia	8,053

The impossibility of gaining any idea as to the amount of urobilin excreted in the stools by an examination of a single twenty-four-hour collection is apparent when the figures for the daily excretion of any of the cases we have worked with are examined (Table 5). The variations, it is seen, are sometimes very large indeed.

To a certain extent, of course, this is accounted for by differences in the quantity of feces passed from day to day, but there are certainly other factors involved. We were particularly struck with the fact that on the days following a period of twenty-four hours during which no stool was passed, there was not as a rule any increase in the urobilin. If

the urobilin in the stools retained in the large intestine were absorbed by the blood-stream, one would have expected an increase in the urobilin in the urine on these days. But on reviewing the figures, no constant increase in the urobilin in the urine could be established. We were then inclined to attribute this apparent loss of urobilin to destruction within the intestine; but when the effect of keeping stools in an incubator at 37 C. was tried, we found to our surprise that there was no less marked loss of urobilin.

TABLE 5.—UROBILIN EXCRETED IN STOOL

Day	Urobilin in Stool			
1	2,408	4,800	16,640	5,120
2	10,880	3,360	5,280	13,440
3	*	3,840	7,680	2,560
4	7,360	960	1,440	*
5	4,480	*	4,800	3,840
6	1,280	6,080	15,360	14,400
7	7,680	10,240	25,920	*
8	4,000	8,000	8,320	35,200
9	*	*	3,840	1,536
10	720	3,200	5,760	3,200
11	7,040	11,200	6,720	7,040

* No stool.

The stools were thoroughly ground up in water and kept in the incubator. Every day, 25 c.c. were removed and extracted with acid alcohol. With the exception of the last stool, the figures show only such differences as lie within the limits of error of the method (Table 6). There was only a slight diminution of urobilinogen and increase of urobilin.[3]

TABLE 6.—EFFECT ON UROBILIN OF KEEPING STOOLS IN INCUBATOR

No.	Fresh Stool	After 24 Hours	After 48 Hours	After 72 Hours	After 96 Hours
1	15	16	16	..	..
2	3	3	2	3	3
3	12	19	14	18	11
4	57	62	65	66	65
5	20	21	20	18	24
6	60	55	48	53	60
7	3	3	3	4	4
8	65	60	55	51	48

So long then as stools are kept from exposure to light there does not seem to be a very rapid loss of urobilin. Nor, on the other hand, is there any increase such as might have occurred if the formation of urobilin in the freshly passed stool had been incomplete.

The absence of great destruction of urobilin in stools kept under these conditions outside the body does not, of course, preclude the possibility

3. Later experiments showed that there was a gradual diminution of both urobilin and urobilinogen, so that after a week about a quarter of the original dilution value had been lost. The greater constancy in the experiment given here was due to concentration by evaporation.

of such an action taking place within the bowel, where absorption and excretion are proceeding and where there is no exposure to air. It was thought that an indication of such a loss of urobilin either by absorption or destruction was obtained when the relative amounts of urobilin present in the contents at different levels of the intestinal tract were compared.

The feces obtained at post-mortem in five cases were ground up in so much acid alcohol as gave, so far as could be seen, an approximately equal consistency. The relative amounts of urobilin were as follows: duodenum, 1; jejunum, 1.2; ileum, 4.8; cecum, 12.3; descending colon, 5.5.

The relative decrease in the descending colon as compared with the cecum certainly suggests a loss of urobilin, but the number of cases examined was too small to permit of any definite conclusion. In all probability, both absorption and destruction are at work. The absence of increased urobilin in the urine on the days of constipation is not by any means conclusive evidence against the occurrence of absorption of urobilin from retained stools, for the absolute want of any relationship between the amount of urobilin in the stools and in the urine is a fact which must impress any one who makes such concomitant observations. No doubt the quantity in the intestine is a factor in determining the amount excreted by the urine, but except under conditions such as an unusually large hemoglobin destruction, when there may be a very great increase of urobilin in the stools, its effect is altogether overshadowed by other influences at work on the urobilin in its passage through the blood-stream, liver, bile, tissues and kidneys.

The interpretation of the results of urobilin estimations in the stools is a comparatively simple matter when compared with the complexities and obscurities surrounding the question of the elimination of urobilin in the urine, and we believe that from a clinical point of view, work on the stools will give results of much greater value and of much less doubtful significance than has been the case up to the present, when the attention of clinicians has been directed almost exclusively to the urine.

Our own experience in cases of pernicious anemia, and the reports of Simpson in cases of malaria have convinced us that, if constipation or diarrhea are avoided, the determination of the average stool urobilin over a period of several days is the best guide we have to the quantity of hemoglobin which is being broken down in the body. Its importance in anemias and in all cases in which excessive blood destruction is suspected does not need to be elaborated.

VII. THE EXCRETION OF UROBILIN IN BILE

Urobilin has been found in human bile post mortem by Jaffé, Müller, Tissier, Gerhardt, Beck and Braunstein. After the introduction of Ehr-

lich's reagent, urobilinogen was found in forty-three of forty-six cases by Kimura. Neither is constantly present, for in cases in which severe diarrhea had occurred, or in which there had been obstruction to the flow of bile into the intestine, they were not found. Austin and Ordway in a very careful study found urobilin present in 94 per cent. of fifty cases with biliary fistulas in which urobilin was present in the stools and urine. In seven cases in which no urobilin could be demonstrated in either the stool or urine, urobilin was present in the bile in two. Fischler found urobilin in the bile of normal dogs. After establishing a biliary fistula and obstructing the common bile-duct, urobilin disappeared from the bile, but it was still found, though in diminished amount, in the stools. When the liver was damaged by amyl alcohol, urobilin appeared in considerable amounts in the bile. He believes that the only way in which this can be accounted for is by assuming that the urobilin was formed in the liver itself.

Our experience with bile removed post mortem goes to show that the amount of urobilin is extremely variable. In Table 7 the value of the urobilin in 10 c.c. of bile is given in sixteen cases.

TABLE 7.—UROBILIN IN BILE REMOVED POST MORTEM

		Dilution Value in 10 c.c. Bile
1.	Pernicious anemia	3,940
2.	Pernicious anemia	80
3.	Pneumococcal meningitis	2,100
4.	Pyonephrosis	4,500
5.	Bronchopneumonia	650
6.	Streptodiplococcal septicemia	0
7.	Cerebral embolism	620
8.	Aneurysm	300
9.	Aortic incompetence	45
10.	Diabetes	200
11.	Bronzed diabetes	1,800
12.	Carcinoma of liver (without duct obstruction)	45
13.	Tetanus	0
14.	Carcinoma of larynx	0
15.	Hypophysis tumor	0
16.	Adherent pericarditis and valvular disease	0

Of these sixteen cases there were five in which no urobilin was found by the method we used. But if larger amounts of bile had been used for extraction we might have found traces, and we therefore cannot say more than that the quantity was at least very small. No relation was found between the length of time between death and examination of the bile and the amount of urobilin found. In one case urobilin to a dilution value of 1,800 for every 10 c.c. of bile was present, although it was removed from the body within three-quarters of an hour after death. It does not seem that post-mortem decomposition of bilirubin can possibly explain the variations in quantity which were found.

On the other hand, there is no obvious connection between the nature of the disease and the amounts obtained. We are inclined to believe that the degree of absorption of urobilin from the intestine just before death is an important factor. In the two patients with pernicious anemia, estimations of the amount of urobilin present in the intestinal contents were made, and an enormous quantity was found in the case which contained so much urobilin in the bile, and comparatively little in the one which showed a low dilution value. In another case in which severe diarrhea had completely emptied the intestine, no urobilin could be detected in the bile. In a dog which died four days after the common bile-duct had been severed and in whose intestines no urobilin was found, there was also no urobilin in the bile, although it was found in the bile of normal dogs. These observations bear out the well-recognized importance of the intestinal source of biliary urobilin, and yet exceptions are sufficiently striking to make one hesitate to accept the exclusively enterogenous origin of urobilin.

In a few cases with biliary fistulas after operations for gall-stones the urobilin in the bile was found to increase as the bile began again to enter the intestine, but there was no definite qualitative relationship between the amount in the bile and in the stools. An exception was found in one patient whose case is discussed later, in whom urobilin was present in large amount in the bile while urobilin was practically absent from the stools. This was a case of gall-stones in which for a period of eight days after a gall-bladder fistula had been established, the total urobilin in the stools amounted to only 720 dilutions, while in the bile an amount equivalent to 35, 144 dilutions was found. The stools contained no more urobilin than we found in a case in which there was undoubtedly a complete occlusion of the common duct. Probably no bilirubin at all was entering the intestine. Later on when the bile again passed through the common duct, the urobilin increased in amount in the stools to 33,216 dilutions in seven days, but the percentage of urobilin in the bile, which still came from the fistula for the first four days of this period, was only 230 dilutions for each hundred c.c. of bile, in contrast with 1,360 when there was practically no urobilin in the stools. In cases of this sort it is difficult to avoid the conclusion that the urobilin has been formed in the liver itself.

VIII. UROBILIN IN SERUM

Urobilin is present in the intestine and is excreted in part by the kidneys. It presumably reaches the kidneys by means of the blood. One would expect to be able to detect it in the blood of those not infrequent patients in whom even more urobilin is found in the urine than in the stools. Yet there is no point in the whole subject on which there is more diversity of results and of opinions. The constant absence of uro-

bilin from the serum in cases of urobilinuria was a main support of the theory advanced by Gilbert and Herscher that urobilin was formed from bilirubin during excretion through the kidneys. Even long after the inadequacy of this hypothesis had been exposed by the work of Müller and the intestinal origin of the urobilin of the urine had been generally accepted, no one was able to demonstrate the presence of urobilin in the blood or serum. Müller and Gerhardt believed that it was not present in appreciable amount in the blood, and Schlesinger reported fifteen cases of very marked urobilinuria in which the serums gave a negative reaction for urobilin.

It was not until 1906 that Huber published a report in which he stated that urobilin was always found in serum when urobilin was present in the urine. He ascribed the negative results of Schlesinger to the fact that he had not added Lugol's solution to the zinc acetate filtrate in order that urobilinogen might be oxidized to urobilin. But considerable doubt was cast on Huber's results when it was pointed out that the green fluorescence characteristic of urobilin might be closely imitated by biliverdin, and that especially after the addition of Lugol's solution, the bilirubin normally present in serum might be oxidized to beliverdin. Biffi (1907) extracted oxalated blood with chloroform, examined the filtrate spectroscopically and found urobilin present in small quantities in a number of cases and stated that in lobar pneumonia it was almost constantly found. Moller could not confirm Biffi's findings as to the relative frequency of a positive reaction for urobilin in serum, but with zinc acetate and Lugol's solution found it in only two cases, both lobar pneumonias, and later with Biffi's method in two other cases, pneumonia and one of pernicious anemia.

Further evidence of the occurrence of urobilin in serum was given by von Jaksch, who stated that in fatal cases of pneumonia the serum separated from blood removed by venesection showed a green fluorescence which disappeared on exposure to light. Conner and Roper (1909), in a study of the relation between the bilirubin in serum and urobilin in the urine, repeatedly and carefully examined the serums of over sixty patients with urobilinuria, with uniformly negative results, until the serum of a patient dying of lobar pneumonia was encountered. This serum gave a markedly positive reaction for urobilin with all the methods. In nine other cases of pneumonia shortly before death the serum contained large amounts of urobilin. In three severe cases of pneumonia in which the patients recovered, urobilin was detected in small amount in the serum, but in fifteen other cases in which the patients lived, no urobilin was found. There was no parallelism between the amount of urobilin in the serum and in the urine.

Lehndorf (1913) could find urobilin in only two of the serums which he investigated. One was from a case of decompensated mitral stenosis and the other from a case of myocardial degeneration with pleurisy shortly before death. Fromholdt and Nersessoff (1912) by a somewhat complicated method and by extracting relatively large volumes of oxalated plasma, were able to demonstrate urobilin in the serum of seven out of eleven cases with urobilin in the urine. They record a doubtfully positive reaction in a case of occlusion of the common duct with no urobilin in either the stools or the urine. The spectroscopic band of urobilin became unmistakable after the addition of iodin, but they are inclined to think that this may possibly have been due to a conversion of bilirubin into urobilin.

Hildebrant (1909), who, after many negative results, found the spectrum of urobilin in the serum in a case of pneumonia shortly before death, believes that urobilin normally circulates in the blood as urobilinogen, because in this case the urobilin band appeared only after the serum had been exposed for some time to light and air. He obtained a red color on mixing Ehrlich's reagent with the serum before the urobilin had appeared. Moller, though he is inclined to agree with Hildebrandt, points out the technical difficulties of urobilinogen recognition in serum, since the hydrochloric acid precipitates the proteins. He obtained an apparently positive reaction in only two of his cases. In three cases Conner and Roper showed the characteristic red color and confirmed the presence of urobilinogen by finding the absorption band of the benzaldehyd compound. But in the other cases in which urobilin was present they did not find any urobilinogen.

Conner and Roper's spectroscopic recognition of urobilinogen is the only observation which can be taken as approaching to a proof of the presence of this body in serum, and in view of the substances of the indol group which may give a spectrum very similar to that of the urobilinogen compound, their statement cannot, in the absence of any data as to the position of the absorption band, be taken as decisive.

In fact, the evidence so far points in just the opposite direction and seems to indicate that urobilin, instead of circulating in the reduction form of urobilinogen, is present as an oxidation product which does not possess the spectroscopic characteristics of urobilin and so escapes detection. This evidence is all the stronger because it has been collected independently by three observers since 1903; none of whom apparently knew about the work of the others. Finding no urobilin in the blood of cases in which he expected it, Schlesinger tried the effect of adding urobilin to serum and to blood. He discovered that whereas it was readily demonstrated in the serum, it disappeared in the whole blood or in serum to which red blood-cells had been added. Clarens made the same

observation and in 1911 Roth and Herzfeld confirmed Schlesinger's experiment and suggested that the disappearance of the urobilin might be due to oxidation.

They found that oxygenated serum destroyed the urobilin and that in whole blood its disappearance might be delayed by carbon dioxid. In a few cases, though not in all, they were able to demonstrate the presence of urobilin in serum after it had stood for from twenty-four to forty-eight hours mixed with finely powdered zinc. Since urobilinogen is found in the urine of patients in whom no urobilin can be found in the serum, it seems that the kidneys must possess the faculty of reducing the oxidized urobilin. They were able to carry out only two experiments with kidney extracts, one of which gave a definite production of urobilin in a serum in which it had previously been found absent. It is noteworthy that urobilin was formed with an alcoholic kidney extract, so that the process cannot depend on any vital activity in the cells.

The conception of the oxidation of urobilin in the blood-stream is strengthened when it is remembered that the only cases in which urobilin has been with certainty found in the serum are those in which there was a great reduction in the available oxygen of the blood. They have been almost without exception cases of lobar pneumonia shortly before death.

The effect on urobilin of the blood and tissues opens up a new field for investigation and it is not improbable that along these lines facts may be discovered which will throw light on the present obscurity.

IX. ANIMAL EXPERIMENTS

The Excretion of Urobilin in Animals

No satisfactory estimations could be made on the stools of rabbits or goats because the amount of urobilin was too small.

The same difficulty, though to a lesser extent, was encountered in dealing with the excreta of cats and dogs. Even when the extract was made as concentrated as possible it seldom required more than a few dilutions to obliterate the spectra of urobilinogen and urobilin. We have not been able to find any systematic investigation of the pigments of the stools of dogs or cats, but it is known that cholecyanin,[5] a derivative of bilirubin which is not regularly present in human stools, is constantly found. Possibly the greater part of the bilirubin in the intestine of these animals may be excreted in other forms than urobilin. Certainly the acid alcohol extracts of the stools contained a large amount of light-absorbing material, so much so as to render the urobilin band somewhat obscure at the low dilution at which it had to be read. And not infrequently other absorption bands were seen. The method did not then give such definite and clear results as were obtained with human stools.

5. Fischler: Inaug. Diss., Heidelberg, 1906, p. 84.

It was soon found that such small amounts of urobilin as were detected varied considerably from day to day. No satisfactory explanation for this variation was obvious, but one possible factor appeared to be the length of time during which the feces remained in the intestine. Hard constipated stools as a rule contained very little urobilin. The following experiment shows how markedly the quantity of urobilin increases during regular action of the bowels as compared with a period of constipation.

Dogs B, C and D were normal. Dog A had had a biliary fistula and a closure of the common bile-duct. At the time of this experiment the fistula was closed and the common duct was again patent. Urobilin was present in the urine and was added to the amount in the stools. The figures (Table 8) represent the total dilution value of the urobilin excreted during ten days. In the first period the dogs were constipated; in the second sufficient cascara was given to obtain a stool every day without giving rise to marked diarrhea.

TABLE 8.—UROBILIN IN CONSTIPATION AND IN REGULAR BOWEL ACTION

	First Period of Ten Days		Second Period of Ten Days	
	Urobilin	Number of Stools	Urobilin	Number of Stools
Dog A	5,640	4	13,120	10
Dog B	14,400	8	19,200	10
Dog C	9,200	5	16,420	10
Dog D	12,860	7	37,200	9

In these four dogs the total urobilin during the period of constipation was 42,100 and in the period during which cascara was given, 85,940, more than twice as much. During another ten-day period castor oil was given, and the total amount passed was 51,250, but regular daily movements were not obtained. Constipation, therefore, in dogs diminishes the amount of urobilin in the stools. We are satisfied that this is true for man also though we cannot bring forward any systematic evidence. The diminution may be due either to increased absorption of urobilin from the intestine or to destruction of the urobilin. If increased absorption were the cause, the excretion of urobilin in the urine should be increased. In normal dogs or cats urobilin was never found in the urine in sufficient amount to estimate. But in Dog A, in which the common duct had been tied and a biliary fistula made, it was found six months later that communication between the bile-ducts and the intestine had become reestablished and that the fistula had closed. In this animal considerable amounts of urobilin were constantly present in the urine. The cause of this was found at death to be an extremely marked cirrhosis of the liver, which had developed probably as a result of infection through the fistula combined with the initial bile stasis.

TABLE 9.—UROBILIN IN URINE AND IN STOOL OF DOG A

Date	Urobilin in Urine	Urobilin in Stool
Dec. 2	160	No stool
Dec. 3	248	800
Dec. 4	192	800
Dec. 5	224	No stool
Dec. 6	21	400
Dec. 7	100	No stool
Dec. 8	160	No stool
Dec. 9	120	No stool
Dec. 10	240	No stool
Dec. 11	400	1,200
Dec. 12	520	No stool
Dec. 13	240	No stool
Dec. 14	280	800
Dec. 15	80	No stool
Dec. 16	200	800
Dec. 17	600	No stool
Dec. 18	160	No stool
Dec. 19	680	400
Dec. 20	480	No stool
Dec. 21	500	No stool

Increased absorption during constipation might be expected to show itself in a rise of urobilin in the urine in this case. Yet we were not able to establish any such increase in urinary excretion. The average amount of urobilin in the urine on the seven days in which urobilin was lost from the body in the stools was 273, and 272 on the thirteen days during which urobilin was retained in the intestine. This evidence would have been more convincing if we had succeeded in contrasting a period of regular evacuation with a period of constipation, but on giving cascara it was found to be impossible to be sure of excluding contamination of the urine with the stools. Still if the figures for the four days from December 3 to December 6, inclusive, during which three stools were passed, are contrasted with those from the 7th to the 10th when there were none, it is seen that a total of 685 dilutions of urobilin were present in the first period, and only 620 when the bowels did not act at all.

It seems probable, therefore, that destruction of urobilin plays a much larger part than increased absorption in the diminution of urobilin which occurs during constipation. This is only what would be expected of a substance which outside the body is so readily altered.

Diarrhea, unless it is so pronounced as to lead to the excretion of unaltered bile, does not seem to have nearly so great an influence in diminishing the quantity of urobilin.

The total excretion of urobilin per kilogram of body-weight in dogs which had been kept under exactly the same conditions and on the same diet for a long time was determined over a period of forty-one days (Table 10).

TABLE 10.—Excretion of Urobilin per Kilogram of Body-Weight

	Weight, Kg.	Dilution Value Per Kg.
Dog C	7.82	6,100
Dog B	9.95	5,220
Dog D	17.80	5,450

Dog A, with cirrhosis of the liver, which was also kept under the same conditions for the same time, and which during that period exhibited no obvious signs of ill health, weighed 4.02 kg. and excreted urobilin with a dilution value of 8,806 per kilogram body-weight. This is an increase of 58 per cent. over the average excretion of urobilin in the three normal dogs. It does not seem probable that an increased hemoglobin metabolism could account for this. It appears much more likely that there was either a diminished destruction of urobilin or an increased formation of urobilin from bilirubin. Constipation was present to a more marked degree in this dog than in the others, so that the increase of urobilin was probably even more marked than the figures suggest. A diminished destruction, if such occurred, must have operated in the body itself. If one of the functions of the liver is the synthesis of urobilin absorbed from the intestine into bilirubin, an explanation of the increase in urobilin excretion is afforded by the supposition that the diseased liver in this case allowed much of the absorbed urobilin to pass through it into the general circulation to be excreted in the urine. But the possibility of the increase in urobilin being due to a formation of urobilin from bilirubin in the diseased liver cannot be excluded. Outside the body we found that urobilinogen and urobilin were produced in considerable amounts in bile left at a temperature of 37 C. And it seems possible that such a breaking down of bilirubin may occur in stagnating bile especially if an infection of the bile passages exists.

In attempting to follow the effect of closure of the common bile-duct in dogs on the urobilin excretion, a difficulty was encountered in connection with the methods we used. In the alcoholic stool extracts a pink color was produced which apparently showed the spectroscopic absorption of urobilinogen. That in reality it was some other substance was shown by the difference in the color reaction with Ehrlich's solution and also by the fact that on exposure to light of the zinc acetate filtrate the absorption band still persisted instead of disappearing. In all probability this substance was indol or some derivation of indol. Von Moracewski found that in alcoholic stools there is an increase in indol which has an absorption band very similar to that of urobilinogen.

To avoid confusion with this substance we have taken no account of the urobilinogen either before or after the closure of the common bile-duct. This makes the total amounts given inaccurate, but our object

was to see whether or not the absence of bile from the intestine would completely stop all excretion of urobilin. As is seen from the two experiments reported below, urobilin disappears for a time, but it afterward returns in diminished though relatively not inconsiderable amount in the stools. In both these cases also urobilin after an interval was found in the urine. The impossibility of obtaining urobilin-free stools in dogs even though all bile is prevented from entering the intestine has been noted by Fischler. Brugsch and Yoshimoto found that urobilin reappeared after an interval in the stools of a dog with biliary fistula.

OBSTRUCTION OF COMMON DUCT

Dog E.—Section of Common Duct Removed

Icterus, acholic stools and bile in the urine appeared. At the post-mortem a distended gall-bladder and ducts were found. No communication with the intestines. A few patches of organized lymph were found on the liver and adhesions round the resected duct but no general peritonitis. Urobilin given as total amounts in periods of six days. (Table 11.)

TABLE 11.—Total Urobilin, Dog E, in Periods of Six Days

Period	Stools	Urine
BEFORE COMMON DUCT CLOSURE		
First	3,200	0
Second	2,320	0
AFTER COMMON DUCT CLOSURE		
Third	0	0
Fourth	512	185
Fifth	32	56
Sixth	320	0
Seventh	160	76

Dog F.—Section of Common Bile-Duct Removed

Uninterrupted recovery with development of jaundice. At the post-mortem the gall-bladder and ducts were found to be greatly distended. There was no communication between them and the intestine. The urobilin is given as a total of estimated dilution value for the stools and urine passed in periods of six days. (Table 12.)

TABLE 12.—Total Estimated Dilution Value of Urobilin, Dog F, in Periods of Six Days

Period	Stools	Urine
BEFORE COMMON DUCT CLOSURE		
First	9,312	0
Second	5,120	0
AFTER COMMON DUCT CLOSURE		
Third	0	0
Fourth	0	0
Fifth	576	396
Sixth	2,368	1,084

BILIARY FISTULA WITH OBSTRUCTION OF COMMON DUCT

In three other dogs the common duct was obstructed and a biliary fistula established. No jaundice developed.

Here also the amount of urobilin in the stools decreased, but did not disappear. Urobilin appeared in the urine of Dog A, which was afterward found to have

developed a cirrhosis of the liver. In the other two it was absent. The urobilin is given as the total dilution values of six-day periods. (Table 13.)

After death no communication was found between the bile-passages and the intestine in Dogs G and H.

TABLE 13.—Total Dilution Values of Urobilin, Dogs G, H and A, in Periods of Six Days

	Period	Stools	Urine*
	BEFORE OPERATION		
Dog G:	First	1,920	0
	Second	1,590	0
	Third	3,524	0

* Urobilin was occasionally found in the urine, but this was due to contamination with bile. It was at first difficult to prevent the urine wetting the dressings and taking up some of the bile which was rich in urobilin.

	AFTER OPERATION		
	Fourth	240	0
	Fifth	1,845	0
	Sixth	560	0
	Seventh	384	0
	BEFORE OPERATION		
Dog H:	First	1,744	0
	Second	930	0
	Third	632	0
	AFTER OPERATION		
	Fourth	64	0
	Fifth	176	0
	Sixth	688	0
	BEFORE OPERATION		
Dog A:	First	3,480	0
	Second	3,256	0
	AFTER OPERATION		
	Third	728	0
	Fourth	520	22
	Fifth	280	68
	Sixth	1,260	26
	Seventh	632	239
	Eighth	720	...

The presence of urobilin in the stools after closure of the common bile-duct cannot be explained as due to the excretion of bilirubin into the intestine from the blood, for it was also found in the stools of the dogs in which the bile was allowed to drain out of the body and in which there was no increase of bilirubin in the blood. The only possible explanation seems to be that urobilin itself was excreted from the blood into the intestine. Where was this urobilin produced? Urobilin is known to have formed in old hematomas in various parts of the body, so that the formation of urobilin from bilirubin in the tissues of the icteric dogs is a possibility. But the fact that it also was found in the excreta of dogs which were not jaundiced points to the liver as the place

of formation, for in the dogs with biliary fistula this was the only organ in which bile was present. Urobilin was found in the bile escaping from the fistulas.

We must conclude then that in dogs the intestine is not the only site of origin of urobilin, but that under certain conditions urobilin is formed in the liver. Whether or not this is a normal function of the liver is another question which will be discussed later.

These experiments, though few in number and inconclusive in their results, are yet sufficient to show the complexity of the subject and the number of factors which may play a part in excretion of urobilin. Variations in the amount of formation of urobilin in the intestine or in destruction after it has been formed, the degree of absorption from the intestine, the fate of the absorbed urobilin in the liver, whether it is rebuilt to bilirubin or is allowed to pass into the general circulation, the question as to a change of bilirubin into urobilin in the liver under certain conditions, and the possibility of the excretion of urobilin from the blood into the intestine are some of the points which have to be considered in interpreting the results.

In any case we must conclude that in dogs at least the theory of the exclusively enterogenous origin of urobilin does not suffice to meet the facts.

X. CLINICAL REPORTS

To enumerate all of the conditions in which urobilin has been found in excess clinically does not seem to us desirable as we wish to point out the significance of its occurrence in the few in which its clinical study has been found to be of most value. The following clinical reports are selected from a series of observations made by us during the last two years.

Diseases of the Liver without Much, if Any, Interference with the Entrance of Bile into the Intestine

TABLE 14.—SUMMARY OF CASES 1-6

No.	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin Urine	Stools
1	I. N.	53	Portal cirrhosis	572	3,647
2	A. F.	10	Portal cirrhosis	241	3,088
3	C. D.	14	Cirrhosis (syphilitic)	456	3,451
4	C. B.	30	Bronzed diabetes	5,800	7,400
5	F. M.	4	Hanot's cirrhosis	2,034	3,360
6	F. O'B.	15	Abscess of the liver	1,713	4,047

CASE 1.—I. N., enlarged liver with ascites. Red blood-cells about 3,000,000 and hemoglobin 55 per cent. Remained during the time of observation in about the same condition. Fluid was removed from the abdomen on several occasions. Urobilin was never found in it. There was a slight grade of jaundice, the serum on one occasion showing six times the normal amount of bilirubin. One month after the estimations were discontinued the patient died and the liver showed a well-marked portal cirrhosis. Chart 8 shows the urinary and stool excretion

of urobilin. The stools were given as averages for periods of three days to diminish variations due only to changes in the motor activity of the colon and rectum. The considerable variations in the quantities in the stools and the fact that urobilin was not constantly found in the urine are the noteworthy points in this case.

CASE 2.—A. F. A typical case of portal cirrhosis.

CASE 3.—C. D. Small liver, large spleen, hematemesis melena. Glycosuria with polyphagia, polydipsia and emaciation. Positive Wassermann.

CASE 4.—C. B. Very much enlarged liver and spleen. Ascites. Pigmented skin. Glycosuria. At post-mertem the diagnosis of bronzed diabetes was confirmed by the finding of a deeply pigmented cirrhotic liver and pancreas. Chart 9 shows the great variations in the amounts of urobilin in the urine.

CASE 5.—F. M., A man aged 48 complaining of persistent slight jaundice of two years' duration. No loss of weight. No pain. General health good. Liver greatly enlarged, extends 10 cm. below costal margin. Spleen much enlarged, projects 12 cm. below costal margin. No ascites. Urine contains bile. Red blood-cells 4,400,000. Hemoglobin 86 per cent. Negative Wassermann.

TABLE 15.—DAILY UROBILIN IN CASE 5

Date	Urine	Stools
November 23	1.300	4,800
November 24	2,950	5,120
November 25	1,104	960
November 26	2,784	2,560

CASE 6.—F. O'B. Admitted with a temperature and polymorphonuclear leukocytosis, complaining of pain and tenderness in the right hypochondriac region. On the eighth day after admission the abdomen was opened and a large abscess of the liver drained.

In this group of cases the urobilinuria is evidently not due to an increased formation of urobilin in the intestine, for the quantities found in the stools are not above the normal limits. There is no evidence of any overflooding of the liver by urobilin derived from an increased destruction of hemoglobin. That the abnormal quantity of urobilin in the urine is to be ascribed to the pathological changes in the liver which were present to a marked degree in all these cases is suggested not only by the constancy of the association of urobilinuria with liver disease which has been noted by all observers, but also by the fact that in particular cases it is possible to observe the diminution and final disappearance of urobilin from the urine in those cases in which an abnormal condition in the liver is removed. The case of F. O'B. with a large liver abscess was a good example of this. During the week preceding the opening of the abscess the average daily excretion was 2,187. For a few days after the operation the quantity in the urine remained high and then dropped so that the daily average for the seven days after operation was only 244.

It will be noted that it is not possible to exclude pronounced disease of the liver by a failure to find urobilin in the urine on one examination. As may be seen from the chart of the case of I. N. (Chart 8, Case 1), who had a very marked cirrhosis of the liver, there was a period of five days during which no urobilin could be detected. And even in the

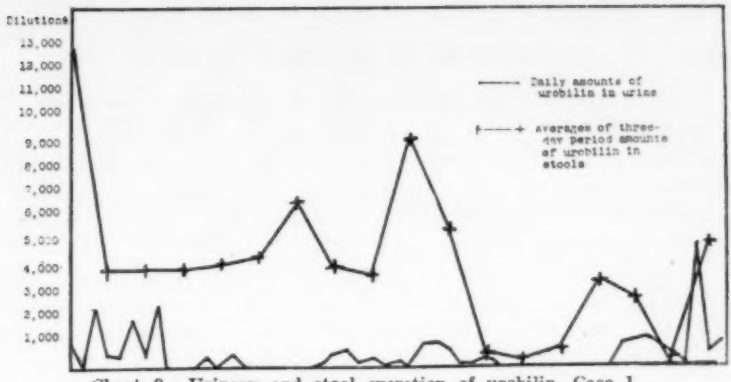


Chart 8.—Urinary and stool excretion of urobilin, Case 1.

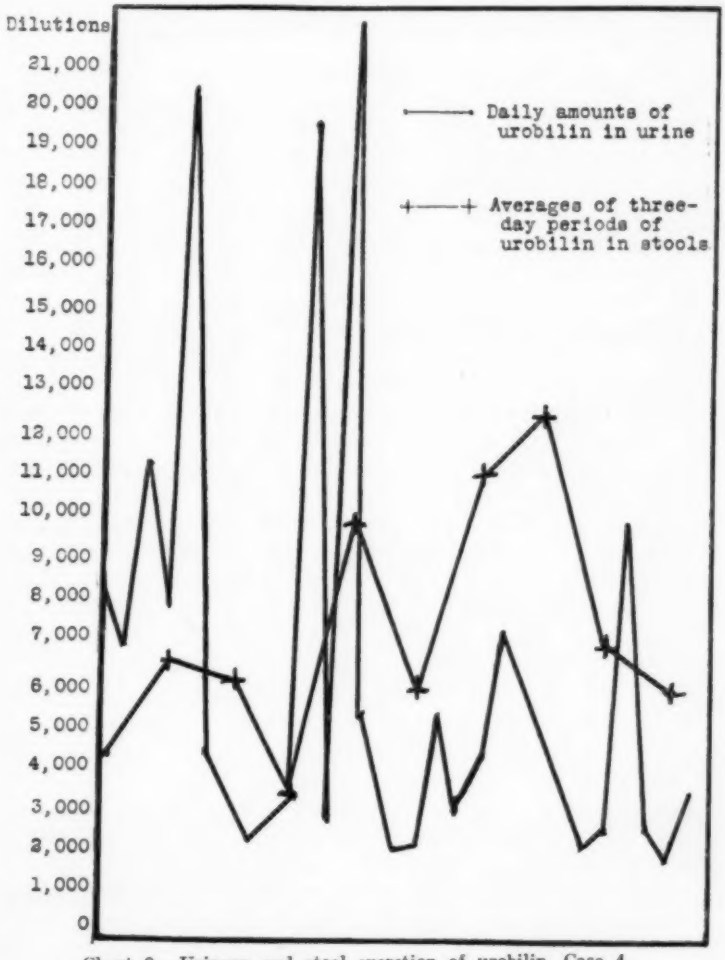


Chart 9.—Urinary and stool excretion of urobilin, Case 4.

patient C. B., in whose urine there was as a rule such a large amount of urobilin, none was found in one twenty-four-hour specimen. The fluctuation in the quantity of urobilin from hour to hour and from day to day makes it imperative to take the average of a series of twenty-four-hour specimens before an idea can be obtained as to the grade of urobilinuria in any particular case.

The study of a large series of cases of liver disease along these lines, with concomitant estimations of the urobilin in the stools and in association with other tests of hepatic efficiency, will be necessary before it is possible to say whether or not the degree of urobilinuria may be taken as an approximate guide to the amount of impairment of liver function.

Cases in Which Part of the Bile Entered the Intestine and Part Left the Body through a Gall-Bladder Fistula

TABLE 16.—SUMMARY OF CASES 7 AND 8

No.	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin		
				Bile	Urine	Stools
7	A. R.	18	Cholelithiasis	788	0	4,240
8	B. H.	13	Chronic cholecystitis	1,505	0	2,669

CASE 7.—A. R. This patient was operated on for relief of dyspeptic symptoms. No icterus. A thickened gall-bladder containing a large stone was found. The gall-bladder was drained. There was no jaundice following the operation.

CASE 8.—B. H. Operated on for symptoms of dyspepsia. No jaundice before or after operation. A moderately thickened injected gall-bladder with adhesions was found and drained.

In these cases there was no obstruction to the flow of bile through the common duct, and only a part of the bile escaped from the fistula.

The urobilin found in this fraction of the bile is of interest in connection with the question as to how much of the urobilin is simply excreted again into the intestine after absorption by the portal blood-vessels. To judge from these two cases, it would appear that a very large part of the absorbed urobilin is excreted into the intestine unchanged. But it must be remembered that neither of these was a normal case, and that the possibility of the formation of urobilin in the liver itself existed.

Complete Obstruction to the Entrance of Bile into the Intestine

TABLE 17.—SUMMARY OF CASES 9-12

No.	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin	
				Urine	Stools
9	F. W.*	34	Carcinoma of hepatic ducts	0	178
10	G. W.	5	Carcinoma of stomach	0	0
11	A. M.	6	Carcinoma of pancreas	0	0
12	B. M.	3	Carcinoma of duodenum	0	0

* Thirty-five gm. of dried bile were given to this patient by mouth in an attempt to confirm Müller's experiment in which he succeeded in producing urobilinuria in such cases. No urobilin appeared in the urine, but this may have been due to the form in which the bile was given. Fromholdt also obtained negative results with various preparations of bile and found that it was necessary to give fresh bile as Müller himself did. Some of the urobilin found may have been derived from bile given by mouth, but it was also found before it was taken and also more than two weeks afterward.

CASE 9.—F. W. This was a case which we have already reported in which there was a large liver, deep jaundice and clay-colored stools. At the post-mortem a large carcinomatous mass in the liver was found to have completely obstructed the hepatic duct.

CASE 10.—G. W. Jaundice had been present for two weeks before the estimations were made. Afterward, at operation a pyloric carcinoma obstructing the common duct was found.

CASE 11.—A. M. Deep jaundice of several months' duration. Post mortem a scirrhous carcinoma of the pancreas was found.

CASE 12.—B. M. At operation, a carcinoma of the duodenum was found which completely obstructed the ampulla of Vater.

In these four cases, no bile was entering the intestine except such traces as might be excreted from the blood. Bile was present in all the tissues of the body, and practically the only mode of excretion was through the urine.

The law of the enterogenous theory of the origin of urobilin, that without the entrance of bile into the intestine, no urobilin is formed, is borne out by the last three cases but not by the first.

In itself, we should not have been inclined to attribute much importance to this exception, if it had not been that Fischler and we ourselves had found urobilin in the stools of dogs with closed common ducts, and that several observers (Gerhardt, Hoppe-Seyler, Tsuchuya and Fischer and Meyer-Betz) have even found urobilin in the urine when no bile was entering the intestine.

Our method gives positive results in the urine only when the amount is distinctly larger than normal, so that urobilin may very well have been present. And it is noteworthy that we found urobilin in the fluid removed post mortem from the broken-down cancer tissue in the liver.

In the other three patients in whom no urobilin could be detected in the stools, the liver itself was not directly involved. It seems, then, that this case gives some evidence in support of the hypothesis that the diseased liver may itself form urobilin.

Cases in Which there was Obstruction to the Entrance of Bile into the Intestine and in Which Part of the Bile Left the Body through a Gall-Bladder Fistula

TABLE 18.—SUMMARY OF CASES 13 AND 14

No.	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Bile	Urobilin Urine	Urobilin Stools
13	A. R.	5	Cholelithiasis	0	0	0
14	E. W.	8	Cholelithiasis	5,856	640	160

CASE 13.—A. R. Admitted with typical signs of acute cholecystitis. Slight icterus but no bile in the urine. The gall-bladder was drained. For the first few days the jaundice deepened and bile appeared in the urine. Thereafter, it diminished and on the fifth day after operation the urine was free from bile. On the seventh day no icterus could be detected.

CASE 14.—E. W. The patient was operated on for symptoms of gall-stones. There was no jaundice. A stone was removed from the common duct and the gall-bladder drained. After the operation there was deep jaundice, acholic stools and bile in the urine.

In both of these patients the manipulations during the operation caused a closure of the common duct, and the drainage through the fistula was not sufficiently free to prevent the occurrence of jaundice. There was, therefore, a complete or almost complete obstruction to the passage of bile into the duodenum combined with the escape of a part of the bile through the gall-bladder fistula.

Though clinically so similar, there is, nevertheless, a remarkable contrast in the urobilin figures. The patient A. R. might be cited as an excellent example in support of the purely enterogenous origin of urobilin, while the patient E. W. appears to offer very strong evidence in support of the possibility of the formation of urobilin in the liver under certain conditions.

The subsequent course of urobilin excretion in these two cases appears to be worthy of mention, and for this purpose it is most conveniently divided into periods corresponding with changes in the biliary excretion.

In Case 13, during the days immediately following the operation, when bile was still present in the intestinal tract, considerable amounts of urobilin were found in the urine and some in the bile. (Table 19, first period.) Thereafter, during the time in which there was jaundice, acholic stools and bile in the urine, urine, no urobilin was found either in the bile or urine. (Table 19, second period.)

In the third period, the jaundice diminished, the stools became colored, and with this urobilin reappeared in the bile and urine.

The fourth period seemed to illustrate the effect of recovery of the liver function, for as the patient's condition improved, the urobilin was found to diminish and at last disappear from the urine in spite of the fact that more bile was entering the intestine because of the removel of the gall-bladder drain.

TABLE 19.—UROBILIN IN CASE 13

Period and Date	Bile	Urine	Stools
First	432	1,270	*
Second	0	0	0
Third	492	1,240	5,896
Fourth:—			
April 1	144	704	0
April 2	18	864	1,600
April 3	†	656	7,680
April 4	...	448	8,960
April 5	...	0	7,680
April 6	...	0	6,400
Fifth:—			
May 1	0	0	160
May 2	0	1,224	240
May 3	0	3,900	64
May 4	84	4,480	‡
May 5	36	7,800	19,540
May 6	120	5,040	6,784
May 7	224	1,700	9,230

* No stools.
† Drain removed.
‡ Stool lost.

Three weeks later the patient returned with recurrence of the cholecystitis and at least a partial blocking of the common duct. On May 1 the fistula was reopened and much bile, mucus and pus escaped. Urobilin, present at first in very small amounts in the stools, increased in the next few days and urobilin reappeared in the urine and bile. (Table 19, fifth period.) During the whole of the next month during which the patient was under observation, urobilin was present in considerable amount in the urine, as if during this second attack, the liver had suffered some more lasting damage. Subsequently, though sometimes found in small amounts, it was often absent.

This case, therefore, supports what may be called the orthodox theory of the necessity of the presence of bile in the intestine for the production of urobilin, and also may be taken as illustrating the effect of damage to the liver on the urinary excretion of urobilin.

On the other hand, Case 14 cannot be reconciled with the exclusively enterogenous origin of urobilin and is the clearest instance we have encountered of the necessity of admitting that, at any rate in some cases, urobilin may be produced elsewhere than in the intestine.

During a time when the stools were colorless, the skin jaundiced, and the urine laden with bile, that is to say when everything pointed to the practical absence of bile from the intestine, there was a very large excretion of urobilin in the bile and a not inconsiderable amount in the urine.

TABLE 20.—UROBILIN IN CASE 14

Period	Bile	Urine	Stools
First	5,856	640	160
Second, (average daily excretion)	1,176	592	3,625

During the next eight days, urobilin reappeared in the stools and the jaundice diminished, but the urobilin in the bile and urine, instead of increasing as one might have expected, decreased in amount.

It seems most probable that in this case the urobilin in the urine during the first period was absorbed by the blood-stream from the urobilin in the bile and excreted through the kidneys. The diminution during the second period, in spite of the reentrance of bile into the intestine, may perhaps be ascribed to a decrease in the formation of urobilin by the liver, as it began to recover its normal functions. In any case, the large amount of urobilin in the bile cannot have been absorbed from the intestines.

If, in such cases as these, a marked urobilinuria may be present in spite of the total or almost total absence of urobilin from the intestine, it would seem to be likely that in all cases of urobilinuria in liver disease, some part at least of the urobilin has an extra-intestinal origin.

CASE 15.—A. H. Pernicious anemia. During the period in which estimations were carried out, the red blood-cells varied from 1,300,000 to 1,800,000 and the hemoglobin from 31 to 38 per cent.

CASE 16.—A. T. Pernicious anemia. The red blood-cells numbered 1,000,000 and the hemoglobin was 28 per cent.

Diseases Associated with Changes in Hemoglobin Metabolism

TABLE 21.—SUMMARY OF CASES 15-19

No.	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin Urine	Stools
15	A. H.	25	Pernicious anemia	470	24,977
16	A. T.	12	Pernicious anemia	1,058	22,014
17	E. P.	4	Secondary anemia from intestinal hemorrhage	0	2,400
18	L. S.	4	Primary polycythemia	1,849	3,240
19	H. L.	7	Secondary polycythemia from asthma and emphysema	806	10,011

CASE 17.—E. P. For eleven years the patient had had bleeding from the bowel. The stools were sometimes tarry, sometimes contained bright red blood. No cause was found even after the abdomen was opened. At the time of observation the red blood-cells varied from 2,100,000 to 2,550,000 and the hemoglobin from 27 to 29 per cent. Hemorrhage ceased after a Whitehead operation and the patient entirely recovered.

TABLE 22.—UROBILIN IN CASE 17.

Date	Urine	Stools
November 23	0	5,120
November 24	0	*
November 25	0	2,560
November 26	0	1,920

* No stool.

CASE 18.—L. S. Noticed a bluish tinge in her face and hands three years ago. During the time of observation the red cells numbered from six and one-half to seven and one-half millions and the hemoglobin was over 110 per cent. No normoblasts were found. There was no splenic enlargement.

TABLE 23.—UROBILIN IN CASE 18

Date	Urine	Stools
September 5	4,455	800
September 6	1,560	8,960
September 7	1,080	3,200
September 8	360	*

* No stool.

CASE 19.—H. L. Asthma and emphysema since childhood. Marked and constant cyanosis. At the time of observation there were 7,350,000 red blood-cells, and 120 per cent. of hemoglobin. There was no fever and the general health of the patient was as good as it ever had been.

TABLE 24.—UROBILIN IN CASE 19

Date	Urine	Stools
September 2	980	8,000
September 3	2,016	14,080
September 4	0	11,520
September 5	864	8,000
September 6	0	9,600
September 7	900	4,480
September 8	880	14,400

Pernicious Anemia.—The amounts of urobilin in the stools of these two patients on whom a careful study was made are much higher than we have found in any other condition, although in neither of them was there any acute blood crisis during the time of observation. The

quantity in the urine was not very great, but in two other patients from whom the stools could not be obtained for examination, very large amounts were found, corresponding to 4,895 and 16,800 dilutions.

That the increase of urobilin in the urine is in the main due to an overburdening of the liver with an excessive amount of urobilin absorbed from the intestinal tract, and not to a primary functional deficiency of the liver, is most probable.

The marked increase of urobilin in the stools is of special interest as evidence of a distinction between those forms of anemia associated with a great increase in hemoglobin metabolism, such as the cases of pernicious anemia we have cited and others, such as the secondary anemias following hemorrhage and carcinoma, in which the low urobilin content of the stools indicates a diminished destruction of hemoglobin.

Secondary Anemia.—It is especially in cases of secondary anemia that combined urobilin estimations in the urine and stools ought to afford interesting and valuable results.

The case of secondary anemia from hemorrhage which is given above affords a striking contrast when compared with the cases of pernicious anemia. Other cases in which secondary anemia was a complication of some other condition such as carcinoma, showed a diminution rather than an increase in total urobilin excretion.

On the other hand, the case of H. D., who had a diplostreptococcal endocarditis, may be cited as an example of an anemia due to increased blood destruction. During the first twenty-six days the average excretion in the stools was 12,841, twice as great as the normal average, and there was a dilution value of 781 in the urine. During the next seventeen days there was a fall in the stool excretion to 7,801 and in the urine to 659, although there was no increase in the percentage of hemoglobin or red blood-cells. The estimation of red blood-cells and hemoglobin can give no adequate guide as to the total turn-over of hemoglobin in the body, but an approximation to this may be obtained by urobilin determinations in the urine and stools. In cases of anemia of unknown origin, the finding of an increased urobilin excretion might be of diagnostic importance in directing attention to the search for some active blood-destroying agent as the cause.

Polycythemia.—The case of primary polycythemia gave unexpected results. The urobilin in the stools was low instead of being increased, and at the same time there was a very considerable amount of urobilin in the urine, although there was no evidence of any liver disturbance. The finding of urobilin in the urine of the patient with secondary polycythemia was also unexpected.

Both these patients were markedly and constantly cyanosed, and when the connection between cyanosis and the presence of urobilin in

the serum is recalled, the question rises as to whether, under normal conditions, a part of the urobilin absorbed from the intestine may not escape the liver and be changed in the blood, except under certain conditions, such as a diminution in the percentage of oxygen, when it might be excreted in the urine.

Unfortunately, we have not had an opportunity of following up this point in other cases with cyanosis.

Miscellaneous Cases

In a large number of patients with various disorders, no pathological increase in the urobilin in the urine was found.

In some, however, who showed no evidence of gross anatomical disease of the liver or of great blood destruction, considerable amounts of urobilin were present in the urine. One of these, a case of alcoholism, is of interest.

The patient was 59 years old and had suffered from attacks of acute gout for forty years. There were large urate deposits round the joints. At the time of observation, he was recovering from a prolonged spell of drinking, and was in a condition of great prostration with complete anorexia and vomiting. There was some edema of the legs. There was no liver or splenic enlargement. A few months later he died suddenly, but the cause of death was not ascertained.

Unfortunately, satisfactory collections of the stools could not be made, but the amount of urobilin appeared to be very low. On one day no urobilin could be detected and on two others amounts of 216 and 1,728 were found.

The urine over a period of nine days showed a daily average of 1,292.

In the absence of any indication of gross liver disease, the most probable interpretation of the appearance of the large amounts of urobilin in the urine in this case is to be found in damage to the functional efficiency of the liver by excessive amounts of alcohol.

In a patient with a huge retroperitoneal sarcoma who was cachectic and feverish, the average urobilin excretion in the urine was 1,545 and 11,893 in the stools. A case of carcinoma of the stomach without any metastases in the liver had an amount in the urine corresponding to 794, and 4,576 in the stools.

These cases are more difficult to interpret than two others in which there were very large masses of cancerous tissue in the liver, in both of which urobilin was found in great excess in the urine.

The patient with cardiac decompensation and swollen liver, whom we mentioned in our first paper, had an average of 2,682 in the urine and 8,619 in the stools.

Three cases of effusion of blood into the tissues were examined, but cannot be said to throw any light on the question as to whether or not

urobilin in the urine may have its origin in the breaking down of hemoglobin outside the vessels. One was a case of lymphatic leukemia with hemorrhages into the pharynx, gums and orbital cavities. There was no urobilin in the urine and only 2,792 in the stools.

In a case of caseous tuberculous peritonitis with a large amount of blood in the peritoneal cavity there was an average urinary excretion of 1,264, and in a large pulmonary hemorrhage in a case of compensated mitral stenosis there was an average of 415 in the urine and 15,106 in the stools. There are too many other possibilities which might account for the urobilin in the urine in the two cases in which it was present. Cases of complete occlusion of the common duct with large hemorrhages are the only ones by which it is possible to decide this point.

A disturbance of liver function is, of course, a possibility which is difficult to exclude in almost any case, but it does not seem justifiable at present to attribute every case of excessive excretion of urobilin in the urine without increase in the stools to this cause alone.

XI. CONCLUSIONS

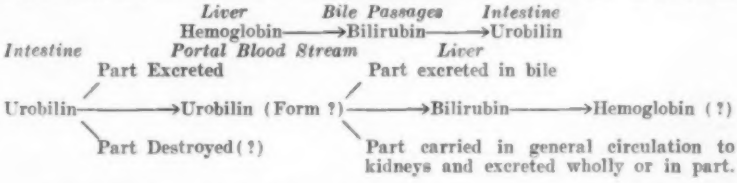
A. General

1. The intestinal formation of urobilin from the decomposition of bile present within the bowel is the usual mode of origin. In complete closure of the common duct due to carcinoma, for instance, there is usually absence of urobilin from the urine, bile and stools.

2. There is evidence that the diseased liver may originate urobilin either directly as a product of its cells, or indirectly from decomposition of bilirubin within the bile passages. Thus, patients in whom no bile is reaching the intestine, may nevertheless show urobilin in large amount in the bile, and to some extent even in the urine and stools if the liver itself is diseased or functionally deficient. The urobilin in the urine and stools of such cases must be due to excretion from the blood of urobilin absorbed from the liver.

The usual sequence in hemoglobin metabolism may be represented as in the accompanying scheme.

SCHEMATIC REPRESENTATION OF USUAL SEQUENCE IN HEMOGLOBIN METABOLISM



On this hypothesis, bilirubin is broken down to urobilin in the intestine and the absorbed urobilin is synthesized in the liver to bilirubin and perhaps by a reverse action again to hemoglobin.

Under abnormal conditions in the liver, formation of bilirubin from the absorbed urobilin from the intestine may fail, and further there may be a reduction of bilirubin to urobilin in the liver itself, a reversal of the normal process.

Under these circumstances, the accumulated urobilin in the liver will be absorbed by the blood-stream and excreted in the urine.

3. An increased quantity of urobilin in the stools indicates increased blood destruction.

In such cases, urobilin may appear in excessive amount in the urine apart from any obvious disease of the liver, because the quantity of urobilin carried to the liver from the intestine being so much increased, the fraction carried past the liver into the general circulation and so to the kidneys is sufficient to lead to an obvious urobilinuria.

4. There is a close association between increase of urobilin in the urine and disturbance of liver function. But even if increased blood destruction is excluded by urobilin estimations in the stools, it is not at present possible to regard the amount of urobilin in the urine as an approximate guide to the functional efficiency of the liver. The occasional occurrence of large amounts of urobilin in the urine of patients in whom there is no reason to suspect damage to the liver, the great and irregular fluctuations of urobilin excretion in cases of liver disease from hour to hour and from day to day, and the want of knowledge as to the fate of urobilin in the blood and tissues make it necessary to recognize that there may be other factors besides the condition of the liver and the amount of blood destruction which influence the excretion of urobilin in the urine.

B. Clinical

Before discussing the clinical importance of the phenomena of urobilin elimination, we should like to draw particular attention, (1) to the insufficiency of our present quantitative methods of urobilin estimation, especially in the urine, and to state that they undoubtedly will be very difficult to overcome because of the marked instability of the group of substances included under this name; and (2) to the value, until now insufficiently appreciated, of estimations of urobilin in the stools as a guide to the amount of blood destruction in the body.

The interpretation of urobilin findings in the urine is interesting, but at present somewhat problematical, whereas the determination of an increased intestinal excretion rests on a much firmer basis, and should yield results of diagnostic importance.

Since this work was undertaken with the primary object of finding out just how much significance should be attached to the presence of abnormal quantities of urobilin and urobilinogen in the excretions in ordinary clinical work, we shall summarize briefly our conclusions, basing

them on our own experimental studies, clinical observations and a fairly exhaustive study of the literature. The difficulty of the problem has been to form a judgment as to how far one is able clinically to make assertions from the data at present obtainable. To choose the essential facts from the maze of non-essential detail and from the numerous observations made to substantiate a previously accepted theory has been difficult. Nevertheless, we feel that we are within safe limits in the following conclusions as to the significance of the presence of abnormal quantities of urobilin in certain pathological conditions.

1. *Hepatic Cirrhosis.*—Urobilinuria is of marked value as evidence of a definite pathological change in the enlarged liver of alcoholic persons and occurs almost constantly in the hypertrophic stage of hepatic cirrhosis. In our cases, we did not find any increase of urobilin in the stools. While in advanced cases, part of the urobilin in the urine may be derived from the formation of urobilin from bilirubin in the liver, it is probable that as a rule it is due to failure on the part of the liver to synthesize to bilirubin, the urobilin brought to it from the intestine.

2. *Hepatic Stasis.*—Estimations of urobilin in the urine are of value in judging the amount of damage done to the liver parenchyma by chronic passive congestion. Hence, a marked increase of it is of ominous prognostic significance in cardiac decompensation.

3. *Jaundice.*—Urobilinuria is absent or insignificant in cases of obstructive jaundice. Its intermittent occurrence points to an incomplete obstruction with concomitant damage to the liver. It is present in the icterus of cases of increased destruction of the red blood-cells. Careful studies for urobilin should be made in suspected cases of chloroform poisoning, acute yellow atrophy and other similar conditions in which the liver is apt to be damaged.

4. *Malaria.*—The great increase of urobilin in the stools and the urobilinuria which occurs in severe cases of malaria is of diagnostic importance in obscure febrile conditions. The persistence of urobilinuria after malarial attacks, when there is no fever and no increase of urobilin in the stools, can be taken as evidence of some complication such as hepatic cirrhosis or abscess.

5. *Anemias.*—By means of urobilin estimations in the stools and urine, those forms of anemia associated with an increased blood destruction may be differentiated. It is probably in this field that the most valuable clinical results will be obtained. The contrast between the very large total urobilin elimination in our cases of pernicious anemia as compared with the small amount found in secondary anemias following hemorrhage or carcinoma was very striking.

6. *Pneumonia.*—A slight increase of urobilin with additional amounts at the time of resolution does not indicate a severe damage of the liver

parenchyma in this disease. It is probably due to a clogging of the liver with the products of resolution and absorbed hemoglobin from the affected lung. A study of the curve of this elimination may be of value in certain pneumonias. Allowances must always be made for other factors such as constipation, diarrhea and the inability of the kidney to eliminate urobilin readily in the presence of a complicating severe nephritis. The early appearance of large quantities of urobilin in the urine in pneumonia, particularly in the presence of jaundice, makes the prognosis grave. The occurrence of urobilin in the serum of pneumonia patients is of the gravest prognostic significance. It is recognized apparently by our present methods only in the presence of a degree of cyanosis markedly unfavorable to the life of the individual.

7. *Carcinoma.*—Except in those cases in which there is practically a complete disappearance of urobilin from the excretions, due to an obstruction of the common duct from carcinoma, we have not found its estimation in carcinomatous cases to be of evident clinical value.

8. *Infections.* — In infectious processes causing parenchymatous changes in the liver or accompanied by hemolysis, urobilin estimations are of definite value in estimating both of these factors in any given case. There is apparently an approximation between the urobilin excretion in the urine and feces and the amount of blood destruction. In such infectious processes as amebic colitis, the appearance and constant presence of urobilin may be of marked value in indicating the presence of inflammatory liver conditions, particularly abscess.

9. *Scarlet Fever, Measles.*—The majority of measles cases present urobilinuria in excess, but, as might be expected, the amount is less and the duration shorter than in scarlet fever with its more definite damage to the hepatic cells.

10. *Decompensation.*—As indicated previously, marked urobilinuria is indicative of pathological change in the liver in the stasis of decompensation. This circulatory stasis calls for the use of increasing amounts of hemoglobin within the body and is associated with a greater production and elimination of bilirubin leading to an inability on the part of the overburdened and congested liver to modify the abnormal amounts of urobilin reaching it. A return of compensation is often indicated by the disappearance of a qualitative urobilinogen or urobilin test. Decompensations in markedly anemic persons are not so apt to be accompanied by increased amounts of urobilin until very profound stasis occurs.

11. *Nephritis.*—Severe nephritis of various types, even in the presence of increased production of urobilin, may prevent definite urobilinuria. Large amounts of urobilin in the urine may be taken as indirect evidence of a certain degree of efficiency in the renal epithelium.

BIBLIOGRAPHY

No attempt has been made to give a complete bibliography, except of the more recent literature. The earlier work is quoted by Hildebrandt (Ztschr. f. klin. Med., 1906, lix, 351), by Fischler (Das Urobilin, Diss. Heidelberg, 1906), and by Lemaire (Thèse de Paris, 1904-1905, xxvi.) Instead of the titles of the papers being given, their bearing on the subject of urobilin has been indicated.

Auché: The Urobilin Spectrum, Comp. rend. Soc. de biol., 1907, lxiii, 711.
A New Method, ibid., p. 713.
Spectroscopic work on bile, Compt. rend., Acad. d. sci., 1908, cxlvi, 496.
Urobilin and Urobilinogen, Compt. rend. Soc. de biol., 1908, lxv, 757.
Urobilin in Bile, ibid., 1908, lxv, 758.
Method of Making Pure Urobilin, Compt. rend. Soc. de biol., 1909, lxvii, 227.
Urobilin in Serum, ibid., 1909, lxvii, 225.
Method of Quantitative Estimation, ibid., 1909, lxvii, 229.

Audibert: Prolonged Catarrhal Icterus, Rev. de méd., 1907, xxvii, 544.

Austin and Ordway: Urobilin in Bile, Boston Med. and Surg. Jour., 1908, clviii, 763.

Baldi: Urobilin and Liver, Lo Sperimentale, 1884, liv, 154.

Bar: Urobilin in Serum, Bull. Soc. d'obst. de Paris, 1903, vi, 149.

Bard: Urobilin in Cerebrospinal Fluid, Soc. de Biol., 1903, lv, 1498.

Bargellini: Urobilin in Typhoid, Lo Sperimentale, 1892, xlvi, 119.

Bauer: Ehrlich's Reagent in Urine and Stools, Zentralbl. f. inn. Med., 1905, xxvi, 833.

Bäumler: Liver Cirrhosis and Urobilin, Deutsch. med. Wchnschr., 1912, xxxviii, 201.

Baumstark: Estimation of Decomposition Products in Urine and Stools by Ehrlich's Reagent, München. med. Wchnschr., 1903, li, 722.

Beck: Formation of Urobilin, Wien. klin. Wchnschr., 1895, viii, 617.

Berggrün and Katz: Urobilin and Tuberculous Peritonitis, Wien. klin. Wchnschr., 1891, iv, 858.

Von Bergmann: Cerebral Lesions, Volk Sammlung klin. Vortr., 190, p. 1541.

Beyer: Trional Poisoning, Deutsche. med. Wchnschr., 1896, xx, 6.

Biffi: Urobilin in Blood, Folia haematol., 1907, iv, 533; Bull. d. Sc. med. de Bologna, 1907, viii, 309.

Bleischroder: Urobilin in Post-Mortem Serum, Berl. klin. Wchnschr., 1909, xlvi, 2, p. 2303; cited by Moller.

Borrien: Hematoporphorin in Meconium, Soc. de Biol., 1910, lxix, 18.

Brauer: Researches on Liver, Ztschr. f. physiol. Chem., 1903-1904, xl, 182.

Braunstein: Urobilin in Stomach Contents, Ztschr. f. klin. Med., 1903, l, 159.

Brissaud and Bauer: Experimental Researches on the Relative Elimination of Bilirubin, Urobilin and Urobilinogen in the Rabbit, Soc. de Biol., 1908, lxiv, 809.

Brugsch and Yoshimoto: Hemoglobin, Bilirubin and Urobilin, Ztsch. f. exper. Path. u. Therap., 1911, viii, 639.

Brugsch and Kawashuma: Ibid., 1911, viii, 639.

Brugsch and Retzlaff: Ibid, 1912, xii, 508.

Brugsch: Hemoglobin and Bilirubin, Fol. haematol., 1910, ii, x, 385.

Brugsch: Folia haematol., 1911, xii, I. T., No. 1, 1911, p. 23.

Cavazza: Urobilin in Urine, II Policlin. Sez. Medica, 1901, viii, 503.

Chaffard: Congenital Icterus, Semaine méd., 1907, xxvii, 25.

Charnas: Preparation and Quantitative Estimation of Urobilin, Biochem. Ztschr., 1909, xx, 401.

Chauffard and Rendu: Urobilin in Feces, Clinical Value, Press méd., 1907, l, 545.

Chase: Urobilin and Liver Conditions, Jour. Am. Med. Assn., 1912, xlix, 329.

Clarens: Theories of Origin of Urobilin, Thèse, Toulouse, 1903.

Clemens: Ehrlich's Reagent in Pathological Urines, Deutsch. Archiv. f. klin. Med., 1901, lxxi, 520.

Connor and Roper: Bilirubin, Urobilin and Urobilinogen, THE ARCHIVES INT. MED., 1908-1909, ii, 532.

De Jager: A Yellow Substance in Urine, Ztschr. f. physiol. Chem., 1910, lxx, 60.

Denigès: Researches on Urobilin, Soc. de Biol., 1897, xlix, 287.

Deroide: Urobilin: Qualitative Test, Soc. de Biol., 1898, l, 302.

Descomps: Methods, Thèse de Paris, 1909-1910, xv.

Dick: Diagnostic Value of Urobilin in Gynecology, Arch. f. Gynäk., 1884, xxiii, 126.

Disqué: Chemical, Ztschr. f. Physiol. Chem., 1878-1879, ii, 259.

Dor: Urobilin in Gasteropods, Soc. de Biol., 1902, liv, 54.

Doyon, Gautier and Policard: Action of Chloroform on Elimination of Urobilin, Soc. de Biol., 1908, lxv, 574; ibid., 1909, lxvi, 616.

Durand: Urobilin, Progrès méd., 1910, xxxviii, 44.

Ehrlich: The Dimethylamidobenzaldehyd Reaction, Med. Wchnschr., 1901, No. 15, p. 151.

Ehrlich and Pröscher: Ehrlich's Reagent, Ztschr. f. physiolog. Chem., 1901, xxxi, 520.

Eichholz: Urobilin and Allied Pigments, Jour. Physiol., 1893, xiv, 326.

Engel and Kiener: Cause of the Brown Color of Urine with Nitric Acid, Soc. de Biol., 1887, iv, 186.

Relation of Urobilin to Icterus, ibid., p. 225.

Falk and Saxl: Relation Between Different Functional Liver Tests, Ztschr. f. klin. Med., 1911, lxxiii, 325.

Filehne: Uselessness of Examination for Urobilin Post-Mortem, Virchows Arch. f. path. Anat., 1890, cxxi, 605.

Fischer: Chemical, Urobilin a Mixture of Substances, Ztschr. f. physiol. Chem., 1911, lxxiii, 204; lxxv, 232.

Fischer: Simple Spectroscopic Recognition of Hemibilirubin (Qualitative), Munchen. med. Wchnschr., 1912, lix, 2555.

Fischer and Bartholomans: Pyrolle Derivatives, Ztschr. f. phys. Chem., 1912, lxxvii, 186.

Substituted pyrolles, Ztschr. f. phys. Chem., 1912, lxxvi, 478.

Fischer and Meyer-Betz: Method of Preparation of Urobilinogen, Ztschr. f. Physiol. Chem., 1911, lxxv, 232.

Fischer and Meyer: Hemibilirubin, ibid., 1912, lxxv, 339.

Fischler: Urobilin, Diss., Heidelberg, 1906.

Fischler: Urobilin, München. med. Wchnschr., 1906, liii, 1784; ibid., 1908, lv, 1421; Deutsche. med. Wchnschr., 1908, xxxiv, 869; Deutsche. Arch. f. klin. Med., 1908, xciii, 426.

Fischler and Bardach: Eck's Fistula and Urobilin, Ztschr. f. phys. Chem., 1912, lxxviii, 435.

Froin: Urobilin in Cerebrospinal Fluid, Gaz. d. hôp., 1903, lxxvi, 1257.

Fromholdt: Urobilin in Rabbits, Ztschr. f. physiol. Chem., 1907, liii, 340.

Urobilin and Hydrobilirubin, Ztschr. f. exper. Path. u. Therap., 1910, vii, No. 3, p. 716.

Urobilin and Reduction in the Intestine, ibid., 1911, ix, No. 2, p. 268.

Fromholdt and Nersessoff: Negative Results in Müller's Experiment Except with Fresh Bile, Ztschr. f. exper. Path. u. Therap., 1912, xii, 400.

Fromholdt and Nersessoff: Urobilin in Blood, Ztschr. f. exper. Path. u. Therap., 1912, xii, 404.

Garrod and Hopkins: Preparation of Urobilin, Jour. Physiol., 1896, xx, 112.

Percentage Composition, ibid., 1898, xxii, 451.

Garrod: Yellow Pigment of Urine, Jour. Physiol., 1897, xxi, 190.

Urinary Pigments, Lancet, London, 1900, ii, 1323.

Gautier: Origin of Urobilin in Feces, Soc. de Biol., 1909, lxvii, 205.

Gautier and Monod: Urobilin in Urine, ibid., 1909, lxvi, 211.

Gautier and Russo: Normal Excretion of Urobilin in Rabbit, ibid., 1908, lxiv, 1026.
Gerhardt: Hydrobilirubin and Relation to Icterus, Diss., Berlin, 1889.
Urobilin, Ztschr. f. klin. Med., 1897, xxxii, 303.
Pathogenesis of Icterus, München. med. Wchnschr., 1905, lii, 889.
Urobilin, Ztschr. f. klin. Med., 1897, xxxii, 303.
Giarré: Clinical, Lo Sperimentale, 1895, xlix, 99.
Gilbert and Herscher: Physiological Stercobilin, Soc. de Biol., 1907, lxiii, 452.
Pathological Stercobilin, ibid., 1907, lxiii, 597.
Formation of Stercobilin, ibid., 1907, lxiii, 802.
Bile in Normal Blood, Presse méd., 1906, xiv, 201.
Stercobilin, ibid., 1908, xvi, 545.
Color of Serum, ibid., 1901, ix, 291.
L'ictère hémaphéique, Presse méd., 1902, x, 1239.
Acholuric Icterus, ibid., 1903, xi, 541.
Renal Origin of Urobilin, ibid., 1902, x, 615.
Grigaut: Urobilin in Blood. ibid., 1909, lxvi, 725.
Grigaut and Monod: Urobilin in Urine, ibid., lxvi, 211.
Grimm: Urobilin in Urine, Arch. f. Path. u. Anat. u. Physiol., 1893, cxxxii, 246.
Graham: Urobilin in Urine After Quinin, Ann. Trop. Med. and Par., 1911, v, 391.
Grimbert: Urobilin and Urobilinogen., Jour. de pharm. et chem., 1911, sér. 7, iii, 425.
Urobilin and Urobilinogen, Soc. de Biol., 1911, lxx, 314, 364; ibid., 1897, lvi, 599.
Günther: Haematoporphyrinuria, Deutsch. Arch. f. klin. Med., 1911, cv, 89.
Harley: Formation of Urobilin, Brit. Med. Jour., 1896, ii, 898.
Herzfeld: Quantitative Estimation of Bilirubin by Spectrophotometer, Ztschr. f. phys. Chem., 1912, lxxvii, 280.
Hess and Saxl: Rôle of Liver in Blood Destruction, Deutsch. Arch. f. klin. Med., 1911, civ, 1.
Hess: Urobilinogen in Scarlet Fever, Med. Klin., 1912, viii, 294.
Hildebrandt: Urobilin and Icterus, Ztschr. f. klin. Med., 1906, lix, 351.
Urobilin, Deutsche. med. Wchnschr., 1908, No. 12, p. 489.
Significance of Urobilin, München. med. Wchnschr., 1909, No. 14, p. 710.
Origin of Urobilin, Deutsch. med. Wchnschr., 1908, lv, 2161.
Urobilin in Serum, München. med. Wchnschr., 1910, lvii, 2574.
Urobilin in Pneumonia, Ztschr. f. klin. Med., 1911, lxxiii, 189.
Hoppe-Seyler: Excretion of Urobilin in Diseases, Virchows Arch. f. path. Anat., 1891, cxxiv, 30.
Formation of Blood Pigments, Ber. d. Deutsch. chem. Gesellsch., 1874, vii, 1065.
Huber: Reason and Meaning of Urobilin, Charité Ann., 1906, xlix, 52
Bilirubin and Urobilin, Med. Klinik, 1910, vi, 56.
Huppert: Analyse des Harns, 1898, p. 533.
Jaffé: Urobilin, Virchow's Arch. f. path. Anat., 1869, xlvii, 405.
Jonas: Urobilinogen in Heart Disease, Zentralbl. f. die ges. inn. Med., 1912, i, 454.
De Jonge (quoted by Simpson): Great Increase of Urobilin in Stools in Malaria.
Justi: Urobilin in Tropical Practice, Zentralbl. f. die ges. inn. Med., 1912, ii, 694.
Hayem: Diagnostic Value of Urobilin, Gaz. d. hôp., 1889, lxii, No. 144.
Hopkins: Urobilin, Guy's Hosp. Rep., 1893, l, 351.
Katz: Clinical, Wien. med. Wchnschr., 1891, xli, 1194, 1295, 1326.
Kayser: Handbuch d. Spektroskopie, 1908.
Kimura: Researches on Urobilin in Bile, Deutsch. Arch. f. klin. Med., 1904, lxxix, 274.

Von Koziczkowski: Clinical Worth of Ehrlich's Reagent, Berl. klin. Wchnschr., 1902, No. 44, p. 1029.
Kunkel: Urinary Pigments, Virchows Arch. f. path. Anat., 1880, lxxix, 455.
Küster: Bile Pigments, Ztschr. f. physiol. Chem., 1906, xliv, 391.
Biliverdin, ibid., 1909, lix, 63.
Hematin, ibid., 1909, lxvi, 164.
Blood Pigments, ibid., 1910, lxvi, 165.
Labbé and Carée: Urobilin in Stools and Urine in Obstructive Jaundice, Soc. de Biol., 1910, lxx, 793.
Ladage: Injections of Urobilin, Thesis Leiden, 1899.
Lehndorff: Bilirubin and Urobilin in Blood-Serum and Serous Fluids, Prag. med. Wchnschr., 1912, xxxvii, 495.
Lemaire: Urobilin, Thése de Paris, 1904-1905, xxvi.
Urohematin, Presse méd., 1904, xii, 644.
Lepehne: Blood-Counts in Cats with Closed Common Ducts, Thesis, Königsburg, 1910.
Leppmann and Husschmidt: Urobilinogen and Inclusion Bodies in Scarlet Fever, Zentralbl. f. inn. Med., 1913, xxxiv, 369.
Lesage: Urobilin in Urine after Poisoning Dogs with Beta-Naphtholin, Soc. de Biol., 1904, lvi, 1026.
Lesieur, Monod and Morel: Urobilin, ibid., 1908, lxiv, 343.
Lesieur and Méret: Is Urobilin Normally Present? Presse méd., 1908, xvi, 501.
Leyko and Marchlewski: Haemopyrole, Biochem. Ztschr., 1908, x, 437; ibid., 1909, xxii, 464.
Levy, Magnus: Formation of Urobilin in Autolysis of Liver, Beitr. z. chem. Physiol. u. Pathol. (Hofmeister's), 1902, ii, 261.
Macfadyen, Nencki and Sieber: Chemical Processes in Intestines, Arch. f. exper. Path. u. Pharmakol., 1891, xxviii, 311.
Marchlewski: Blood Pigments, Ztschr. f. physiol. Chem., 1909, lxi, 276.
Meinel: Origin of Urobilin, Zentralbl. f. inn. Med., 1903, xxiv, 321.
Formation of Urobilin in Stomach, ibid., xxiv, 441.
Minkowski: Pathogenesis of Icterus, Ztschr. f. klin. Med., 1904, lv, 34.
Moller: Origin of Urobilin, Berl. klin. Wchnschr., 1909, xlv, 1639; ibid., 1909, xlvi, 2303.
Monges: Origin of Fecal Urobilin, Soc. de Biol., 1908, lxvii, 609.
Mongour: Urobilin in Serum, Sem. méd., 1908, xxviii, 456.
Mongour and Zellmeyer: Urobilin in Serum (Sylabba's Method), X Congrès franç. de méd., 1908, p. 211; Bull. méd., 1908, xx, 816.
Mongour and Chevrier: Uselessness of Grimbert's Method, Soc. de Biol., 1911, lxx, 664.
Monod: Origin of Urobilin, Arch. de l'app. dig. et de la nut., 1909, iii, No. 5, p. 366.
Moracewski: Quantitative Estimate of Urobilin in Stools by Spectroscope, Arch. f. Verdauungskrankh., 1908, xiv, 378.
Morel and Monod: Method, Soc. de Biol., 1908, lxiv, 205.
Mosse: Polycythemia with Urobilin in Serum, Deutsch. med. Wchnschr., 1907, xxxiii, 2175.
Müller, F.: Icterus, Ztschr. f. klin. Med., 1887, xii, 45; Ztschr. f. Biol., 1884, xx, 327; Proceedings "Innere Medizin," 11th Congress, 1892, p. 118.
Muir and M'Nee: Anemia Produced by Lytic Serums, Jour. Path. and Bacteriol., 1912, xvi, 410.
Münzer and Bloch: Clinical, Arch. f. Verdauungskrankh., 1911, xvii, 260.
Mya: Method, Ann. des Mal. Gén. Urin., 1891, ix, 682.
Nencki and Zaleski: Experimental and Chemical, Ber. d. deutsch. chem. Gesellsch., 1901, xxxiv, 997.
Nencki and Sieber: Production of Urobilin from Hematoporphyrin, Arch. f. exper. Path., 1888, xxiv, 430.
Neubauer: Ehrlich's Reagent, München. med. Wchnschr., 1903, l, 1846.

Neubauer: Sitzungsberichte d. Gessel f. Morph. u. Physiologie, München, 1903, ii, 32.
Neubauer: Injection of Hematoporphyrin, Arch. f. exper. Path. u. Therap., 1900, xliii, 456.
Pel: Relation of Spleen to Bile Formation, Deutsch. Arch. f. klin. Med., 1912, cvi, 250.
Perl: Urobilinuria, Inaug. Diss., Berlin, 1912.
Pick: Toluylendiamin-Icterus, Wien. klin. Wchnschr., 1892, xlii, 307.
Blood-Pigments, Liebig's Ann., 1909, ccclxvi, 237.
Constitution of Blood Pigments, ibid., 1910, ccclxxvii, 314.
Piloty and Merzbacher: Haematopyrollacid, Ber. d. d. chem. Gesellsch., 1909, xlii, 3253.
Splitting of Hematoporphyrin, ibid., p. 3258.
Piloty and Quitman: Constitution of Hemopyrolle, ibid., p. 4663.
Pröscher: Ehrlich's Reagent, Deutsch. med. Wchnschr., 1903, xxix, 927.
Queirolo and Benvenuti: Icterus, Policlinico, 1900, vii, 374.
Rach and Reuss: Urobilin in Measles, Ztschr. f. Kinderheilk., 1912, ii, 460.
Rank: Urobilin, München. med. Wchnschr., 1902, xlix, 1620.
Roth and Hersfeld: Urobilin in Blood, Deutsch. med. Wchnschr., 1911, xlvi, 2129.
Renvers: Urobilin from Infarct of Lung, Deutsch. med. Wchnschr., 1892, xviii, 254.
Rhode: Ehrlich's Reagent, Ztschr. f. physiol. Chem., 1905, xliv, 161.
Rocher: Urobilin in Retention Icterus, Thése de Paris, 1911, xxxix, No. 406.
Rubin: Urobilin in Typhoid, München. med. Wchnschr., 1907, No. 11, p. 507.
v. Rzentkowski: Bile, Biochem. Ztschr., 1909, xvi, 146.
Saillet: Urobilin in Normal Urine, Rev. de Méd., 1897, xvii, 109.
Salkowski: Virchow's Arch. f. path. Anat., 1887, cix, 358.
Recognition of Urobilin and Bilirubin in Feces, Berl. klin. Wchnschr., 1897, xxxiv, 353.
Scheel: Bilirubin in Blood, Ztschr. f. klin. Med., 1912, lxxiv, 13.
Schlesinger: Qualitative Test for Urobilin, Deutsch. med. Wchnschr., 1903, xxix, 561.
Schmidt: Urobilin, Arch. f. exper. Path. u. Pharmakol., 1906-1907, lvi, 130.
Schmidt, Ad.: Hydrobilirubin, Verhandl. der Kong. f. inn. Med., 1895, xiii, 320.
Ehrlich's Solution for Quantitative Estimation of Indol, München. med. Wchnschr., 1903, l, 721.
Simon: Discussion on Ehrlich's Reagent, Am. Jour. Med. Sc., 1903, cxxvi, 471.
Simpson: Urobilin in Stools, Quantitative Relation to Hemoglobin, Biochem. Jour., 1911, v, 378.
Hemoglobin Metabolism in Malaria, Ann. Trop. Med. and Parasitol., 1910, iv, 313.
Case of Blackwater Fever (Ross, Thompson and Simpson), Ann. Trop. Med. and Parasitol., 1910, iv, 307.
Hemoglobin Metabolism in Malaria, Proc. Roy. Soc., 1910, lxxxiii, H. B., p. 562, 174.
Soucek: Renal Origin of Urobilin, abstr. Zentralbl. f. inn. Med., 1906, xxvii, 684.
von Starch: Urobilin Content in Anemia of Children, Jahrb. f. Kinderheilk., F. ii, Erganz., 1900, p. 421; lii, F. ii, p. 421.
Staunig: Coincidence of Urobilin and Albumin in Cardiac Disease, Zentralbl. f. d. ges. Inn. Med., 1912, i. 509.
Steensma: Urobilin in Stools, Nederl. Tijdschr. v. Geneesk., 1907, i, 273.
Qualitative Test for Bilirubin, Biochem. Ztschr., 1908, viii, 209.
Stern: Function Tests of Liver, Deutsch. med. Wchnschr., 1905, xxxi, 1104.
Stich: Urobilin in Exudates, München. med. Wchnschr., 1901, xlviii, 1751.
Strauss: Methods, Urobilin in Serum, München. med. Wchnschr., 1908, lv, 2537.

Surveyor: Clinical, Biochem. Jour., 1908, iii, 439.
Syllaba: Urobilin in Serum, Fol. haematol., 1904, i, 636.
Urobilin in Serum, Fol. haematol., bd. I, Ref. 33, p. 283.
Urobilin in Serum, Deutsch. med. Wchnschr., 1912, xxxviii, 900.
Tarchanoff: Effect of Hemoglobin on Bile Secretion, Arch. f. d. ges. Physiol., 1874, ix, 53.
Tefig and Ibrahim: Urobilin in Urine, Ztschr. f. Urol., 1909, iii, 703.
Thomas: Urobilinogen, Diss., Freiburg, 1907.
Troisier: Hemolysis and Urobilin, Soc. de Biol., 1909, lxvi, 739.
Urobilin in Blood in Icterus, ibid., lxvii, 46.
Parenteral Origin of Urobilin from Hemoglobin, Thése de Paris, 1910, No. 429.
Triboulet: Reduction of Bilirubin to Urobilinogen by Lymphoid Tissue, Soc. de Biol., 1910, lxix, 345.
Tsuchiya: Parenteral Origin of Urobilin, Arch. f. exper. Path. u. Therap., 1910, vii, 352.
Ury: Ehrlich's Reaction in Stools, Zentralbl. f. inn. Med., 1906, xxvii, 40.
Viglezio: Spec. Visibility of Urobilin, Lo Sperimentale, 1891, xlv, 225. (Quoted by Simpson.)
Ville: Action of Palladium Hydrogen on Bile, Soc. de Biol., 1910, lxix, 419.
Weil, Moul and Policard: Two Pigments in Extracts of Stools of Infants, Soc. de Biol., 1911, lxx, 581.
Wilbur and Addis: Urobilin, Jour. Am. Med. Assn., 1912, lix, 929.
Zaleski: Urobilin not Identical with an Oxidation Product of Haemopyrolle, cit. in Mal's Jahresber. de Tierchem., 1905, xxxv, 406; 1906, xxxvi, 455.
Zoja: Hemoglobin and Urobilin, Arch. Folia Haematol., 1910, x, 225.
Zoja: Hemoglobin Decomposition and Urobilinogen, Folia haematol., 1911, xii, 1.

THE EFFECT OF HEAT ON BLOOD-PRESSURE *

L. H. NEWBURGH, M.D.,† AND C. H. LAWRENCE, M.D.
BOSTON

In most infectious disease there is a progressive fall in blood-pressure during the course of the infection. This fall has been attributed to a weakness or insufficiency of the circulatory apparatus. In 1899 Romberg, Pässler, Bruhns and Müller[1] investigated the cause of this presumed weakness by means of animal experiments. They reached the conclusion that the myocardium was not at fault but that the toxin of the infection caused a grave injury to the vasomotor center in the medulla, and that the low pressure was due entirely to an abeyance of the function of this center. Since that time it has been generally held by clinicians that the height of the blood-pressure in the infectious diseases was a measure of the tone of the vasomotor center, that low readings were evidence of the failure of the center and were an absolute indication for therapeutic procedures which stimulated the center.

During the past year one of us (L. H. N.) has been studying, at the Massachusetts General Hospital, the therapy of the circulatory disturbances in the acute infections based on these principles. The results, on the whole, have been such that it seemed desirable to reinvestigate this phase of the problem.

Increased body temperature is a symptom of all infections. Romberg and Pässler[1] failed to eliminate the effect on the blood-pressure of this constant factor. It therefore remains undecided whether the hypotension observed by them was due to toxins, fever, or both.

In the experiments on which this paper is based the effect of increased body temperature, in the absence of infection, was studied in order that its importance as a factor in causing the lowered blood-pressure of infection might be determined.

Halliburton in 1901[2] pointed out that all tissues contain a protein which coagulates between 45 and 50 C. (113 and 122 F.), that during

---

* From the Department of Medicine and the Laboratory of Physiology, Harvard Medical School.

* Submitted for publication Oct. 6, 1913.

† Working under the Dalton Scholarship, Massachusetts General Hospital.

1. Romberg, Pässler, Bruhns and Müller: Experimentelle Untersuchungen über die allgemeine Pathologie der Kreislaufstörung bei acuten Infectionskrankheiten, Arch. f. klin. Med., 1899, lxiv, 652.

2. Halliburton, W. D.: Chemical Side of Nervous Activity, Croonian Lectures, London, 1901, p. 42.

heat rigor the first shortening of mammalian muscle occurs at 47 C. (116.6 F.), and that it loses its irritability—that is, is physiologically dead —at that point. Marinesco[3] has shown that a temperature at 45 C. (113 F.) kills animals in from one to two hours, and 43 C. (109.4 F.) in a longer time; and that the occurrence of death is coincident with the breakdown of the nerve-cells. Halliburton[3] found that saline extracts of brains kept at a temperature of 44 C. (11.2 F.) show a precipitation of globulin after two hours. When the temperature is 42 C. (107.6 F.), the precipitation occurs in from three to four hours. A temperature of from 40 to 41 C. (from 104 to 105.8 F.) causes no coagulation after eight hours. Thus we see that degrees of hyperthermia occasionally seen in infections cause morphological changes in nervous tissue, and that lower temperatures produce effects identical with higher ones if the former obtain over longer periods of time.

One of the factors which is necessary to maintain an efficient blood-pressure is a constant constriction of the arterial tree. To maintain this constriction there must exist not only an active nervous system, but also smooth muscle capable of responding normally to stimuli. The relaxing effect of heat on smooth muscle is well known and must be taken into account when considering the effect of heat on blood-pressure. It has recently been noticed by clinical observers[4] that the blood-pressure is lowered during the application of heat in the form of hot baths or similar maneuvers in nephritis, etc. It is not known whether the body temperature was raised as a result of this form of treatment.

Our experimental procedure was as follows: Rabbits and cats were anesthetized with urethane (ethyl carbamate) employed in a dosage of 2 gm. per kilogram, given by stomach-tube. Occasionally an additional gram of urethane was required in order to obtain complete anesthesia. Blood-pressure was written from the carotid artery by means of a mercury manometer. The animals were placed in a copper tank which contained sufficient water at body temperature to cover the animal except its head. The water was heated by means of an insulated electric resistance-coil in the bottom of the tank. The temperature of the water and the animal's rectal temperature were recorded. Fourteen experiments were performed.

In the first group of experiments the temperature of the animals was rapidly raised. The first effect of the heat was a progressive rise in blood-pressure. At a temperature of between 108 and 110 F., varying

3. Marinesco: Quoted by Halliburton.

4. Lawrence, C. H.: The Effect of Pressure-Lowering Drugs and Therapeutic Measures on the Systolic and Diastolic Pressures in Man, THE ARCHIVES INT. MED., 1912, ix, 409.

Fig. 1. Experiment 10.—The upper line is the carotid blood-pressure; the lower line is 50 mm. Hg pressure. *A* shows the pressure at the beginning of the experiment. The record of the next fifty minutes is omitted. *B* is the tracing obtained from the fiftieth to the sixtieth minute. The cat's temperature was rising from 103.7 to 104.5 F. The temperature of the water was 107.6 F. The respiration was too rapid to count accurately—about 400 per minute—shallow, panting, but still regular. At *C* the wide variations occurring toward the end of the second hour—the one hundred and fifth to the one hundred and twentieth minute—are shown. The cat's temperature was from 108 to 108.5 F. The temperature of the water was 109 F. The respiration was more than 400 per minute, shallow and irregular, but the mucous membranes were pink. *D* represents the last forty-five minutes of the experiment—from the one hundred and thirty-fifth to the one hundred and eightieth minute. It showed a steady fall in pressure broken only by the response to asphyxia at *X*. The small amplitude of the pulse is noteworthy. During this period the cat's temperature rose slowly from 108.9 to 110 F. The temperature of the water was 109 F. During the third hour the respiratory rate has been slowly decreasing and had fallen to 120 per minute just before the effect of asphyxia was tried. It was constantly irregular.

with the individual, the pressure began to fall and continued to do so until the end of the experiment. Experiment 10 is cited in detail as an example of this group.

EXPERIMENT 10.—July 28, 1913, a female cat weighing 4 kg. was given 8 gm. of urethane by stomach-tube at 10 a. m. A cannula was inserted into the left carotid artery, and a cannula into the trachea. A thermometer was inserted as high as possible into the rectum. The animal was transferred to the tank containing water at a temperature of 99.5 F. at 10:45 a. m. A continuous record of the blood-pressure was written. Table 1 shows the temperature of the water, the rectal temperature of the cat, the blood-pressure level and the rate and character of the respiration, marked off in approximately five-minute periods throughout the experiment.

TABLE 1.—RECORD OF EXPERIMENT 10

Time, A. M.	Temp. Water, F.	Temp. Cat, F.	Blood-Pressure mm. Hg	Respiration
10:55-11:00.....	99.7	100.0	155	66
11:00-11:03.....	100.4	100.2	150	94
11:09-11:15.....	103.0	101.0	150	160
11:15-11:20.....	104.0	101.0	144	160
11:20-11:25.....	105.8	101.9	150	260; shallow, panting.
11:25-11:30.....	107.6	102.0	155	400; regular, panting.
11:30-11:35.....	107.6	103.0	162	400; regular, panting.
11:35-11:40.....	107.6	103.7	174	400; regular, panting.
11:40-11:45.....	107.6	104.5	174	400; regular, panting.
11:45-11:52.....	107.6	104.5	*	400; regular, panting.
11:52-12:00.....	.....	106.4	126	400; irregular.
P. M.				
12:05-12:25.....	107.6	†		
12:25-12:30.....	108.6	107.5	110-136	400; irregular.
12:30-12:35.....	109.0	107.5	88-142	Too rapid to count.
12:35-12:40.....	109.0	108.0	90-148	Too rapid to count.
12:40-12:45.....	109.4	108.2	70-140	Too rapid to count.
12:45-12:50.....	109.4	108.2	100-120	Too rapid to count.
12:50-12:55.....	109.4	108.2	88-112	Very irregular.
12:55- 1:00.....	109.4	108.5	110	Very irregular.
1:00- 1:05.....	109.4	108.9	124	Slower.
1:05- 1:10.....	109.4	109.1	117	Slower.
1:10- 1:15.....	109.4	109.6	106	Slower.
1:15- 1:20.....	109.4	110.0	96	Slower.
1:20- 1:25.....	109.0	110.0	86	120
1:27	109.0	110.0	84-154	Asphyxia reaction.
1:30- 1:35.....	109.0	110.0	70-100	66; deep; regular.
1:35- 1:40.....	109.0	110.0	67	52-36; deep; cyanosis.

* Clot removed.
† Clot. Cannula in right carotid.

The animal's temperature was raised from 100 to 104.5 F. in the course of the first hour. The pressure during this period rose from 144 to 174 mm. Hg. During the second hour the temperature rose from 104.5 to 108.2 F. The pressure showed wide variations with a general downward tendency. The heart had become markedly irregular and the steep falls in pressure are evidence of this condition. Respiration became too rapid to count — more than 400 per minute — irregular and shallow. During the third hour the temperature rose from 108.2 to 110 F. The pulse had become very small but more regular. There was a

progressive fall of blood-pressure to 64 mm. Hg. The respiratory rate slowly decreased but its character remained the same. At X (Chart 1) a rubber tube 1½ feet long was attached to the tracheal cannula. As is well known, asphyxia is one of the most powerful stimulants to the vasomotor center. The pressure rose from 84 to 154 mm. Hg — a response of 70 mm. The respirations became deep, regular and slow, falling to thirty-six per minute.

It may well be questioned whether the fall of pressure was not a result of great interference with the function of living tissue brought about by the extreme heat. In favor of this view we have the great cardiac irregularity and the very rapid, irregular respiration, which then becomes slower with increasing heat. On the other hand, the asphyxial rise in pressure indicates that the vasomotor reflex was still intact. But since it was primarily our object to investigate the effect of degrees of heat which are the rule in infections, we performed further experiments in which the heat was not allowed to become excessive. Experiments 7 and 11 are cited as examples of this group.

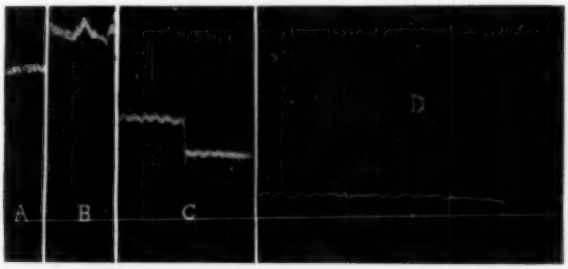


Fig. 2, Experiment 7.—The upper line is the carotid blood-pressure. The lower line is 50 mm. Hg pressure. *A* is the pressure at the beginning of the experiment. The cat's rectal temperature was 100 F. At *B* the pressure is shown forty-five minutes from the beginning of the experiment. The cat's rectal temperature was 104.5 F., that of the water 105.8 F. Respiration was 260 per minute. At *C* the pressure one hour, and one hour and ten minutes, from the beginning of the experiment is shown. The cat's temperature was from 104.5 to 105.3 F., the temperature of the water between 105.8 and 106.8 F. The respiration had fallen progressively from the beginning of the experiment and was now between 176 and 208 per minute. The pressure had fallen 92 mm. during the previous twenty-five minutes and was 62 mm. lower than at the beginning of the experiment. The cat's temperature remained between 105.5 and 106 F. for the next hour and twenty minutes, at the end of which time the respiration, which had become steadily slower, suddenly ceased. The pressure fell slowly during this period from 96 mm. to 65 mm. *D* represents the last ten minutes of the experiment.

Experiment 7.—July 19, 1913, a male cat, weighing 4.2 kg. was given 8 gm. of urethane by stomach-tube at 9:20 a. m. A cannula was inserted into the carotid artery and a thermometer inserted as high as possible into the rectum. At 10:15 a. m. the animal was placed in the tank containing water at a temperature of 99.5 F. For the next forty minutes the water was rapidly heated until

it reached 105.8 F. During the rest of the experiment it was kept between 105 and 106.8 F. Table 2 shows the temperature of the water, the retal temperature of the cat, the blood-pressure level and the rate and character of the respirations throughout the experiment. The time notations in the first column represent periods when the drum was revolving.

TABLE 2.—RECORD OF EXPERIMENT 7

Time, A. M.	Temp. Water, F.	Temp. Cat, F.	Blood-Pressure mm. Hg	Respiration
10:17-10:20.....	99.5	100.0	158	280
10:35-10:38.....	104.0	102.0	176	260
10:58-11:01.....	105.8	104.5	188	260
11:15-11:18.....	105.8	104.5	122	208; shallow, irregular.
11:28-11:31.....	106.8	105.3	96	176; shallow, irregular.
11:35-11:40.....	106.2	105.5	90	176; shallow, irregular.
11:40-11:45.....	106.2	105.5	88	176; shallow, irregular.
11:45-11:50.....	105.8	105.5	84	144; shallow, regular.
11:50-11:55.....	105.8	105.5	84	144; shallow, regular.
P. M.				
12:00-12:05.....	106.1	105.5	74	144; shallow, regular.
12:05-12:10.....	106.1	105.5	74	144; shallow, regular.
12:10-12:15.....	106.1	105.5	72	144; shallow, regular.
12:15-12:20.....	106.1	105.5	72	144; shallow, regular.
12:20-12:30.....	*			
12:30-12:35.....	106.7	105.5	65	144; shallow, regular.
12:35-12:40.....	106.7	106.0	65	111; shallow, regular.
12:40-12:45.....	105.8	106.0	65	100; shallow, regular.
12:46	105.8	106.0	65-0	64; shallow, regular.
12:48	105.8	106.0	...	Ceased.
12:50	†			

* Clot removed.
† Heart stopped.

EXPERIMENT 11.—July 31, 1913, a male cat weighing 4 kg. was given 8 gm. of urethane at 9:15 a. m. by stomach-tube and 1 gm. at 10:15 a. m. A cannula was inserted into the left carotid artery. The left cervical vagus trunk was dissected free and cut. A thermometer was inserted high into the rectum and the animal placed in the water at a temperature of 99.5 F. The central end of the vagus was then stimulated with an induction current of increasing strength until something greater than the threshold value had been reached. This strength of current was used throughout the experiment. The water was now rapidly heated to 104 F. and kept at this temperature for the next eight and one-half hours. The blood-pressure was written approximately once an hour during this period. After the drum had been revolving for several minutes the central end of the vagus was stimulated with the induced current. The nerve was then carefully replaced between the tissues to be kept fresh. Several times the nasal mucous membrane was stimulated.

The group of experiments of which 7 and 11 are types show a well-marked fall in blood-pressure due to a degree of hyperthermia not exceeding that encountered in infections. In all cases the fall occurred before any organic changes due to hyperthermia could be expected to

occur. As is seen in Experiment 12, which was performed as a control, no such fall occurred when the animal, kept at a normal temperature, was subjected to all other conditions of the experiment.

Fig. 3, Experiment 11.—The top line is the carotid blood-pressure. The next line is 0 mm. Hg pressure. Portions of the record from the beginning of the experiment, three hours later, and eight and one-half hours later, are shown; 1, *A*, is the initial level of blood-pressure, 136 mm.; 2, *A*, is the response to vagus stimulation when the pressure is 136; the pressure fell 40 mm.; 1, *B*, is the blood-pressure level, 114, after three hours; 2, *B*, is the response to vagus stimulation when the pressure is 114; the pressure fell 9 mm.; 3, *B*, is the response to vagus stimulation when the pressure is 128; the pressure fell 32 mm.; 1, *C*, is the blood-pressure level, 72, after eight and one-half hours; 2, *C*, is the response to vagus stimulation when the pressure is 70; the pressure fell 7 mm.; 3, *C*, is the response to vagus stimulation when the pressure is 80; the pressure fell 17 mm.; 4, *C*, is the response when the pressure is 84; the pressure fell 29 mm.

TABLE 3.—Record of Experiment 11

Time, A. M.	Temp. Water, F.	Temp. Cat, F.	Resp.	B.-P. mm. Hg	Response to Stimulation
11:20-11:33 A. M.	98.6	99.5	48	136	Vagus 10 sec. 136— 96 = 40
12:25-12:35	104.0	102.5	214	114	Vagus 1 sec. 113—110 = 3
					Vagus 3 sec. 116—107 = 9
					Vagus 10 sec. 110— 99 = 11
					Vagus 10 sec. 128— 96 = 32
1:25- 1:35	104.0	103.2	150	118	Vagus 10 sec. 119—115 = 4
					Vagus 10 sec. 114— 99 = 15
					Vagus 10 sec. 130—112 = 18
					Vagus 10 sec. 130—103 = 27
2:25- 2:35	104.0	103.0	150	114	Vagus 10 sec. 114—105 = 9
					Vagus 10 sec. 128— 96 = 32
3:25- 3:30	104.0	103.2	106	104	Vagus 10 sec. 105— 97 = 7
4:35- 4:40	104.0	103.2	106	100	Vagus 10 sec. 98— 85 = 13
5:35- 5:40	103.6	103.2	106	87	Vagus 10 sec. 88— 74 = 14
					Nose 10 sec. 88—109 = 21
6:40- 6:45	103.6	103.2	100	76	Vagus 10 sec. 78— 62 = 16
					Nose 10 sec. ? ?
7:40- 7:53	104	103.2	100	72	Vagus 10 sec. 70— 63 = 7
					Vagus 10 sec. 79— 62 = 17
					Vagus 10 sec. 84— 64 = 20

EXPERIMENT 12.—Aug. 6, 1913, a male cat weighing 4.2 kg. was given 8 gm. of urethane by stomach-tube at 9 a. m., 1 gm. at 9:30 a. m., and 1 gm. at 9:55 a. m. A cannula was inserted into the left carotid artery, and a thermometer high into the rectum. The cat was placed in a tank containing water at 100.4 F. at 10:15 a. m. The cat's temperature fell from 100.4 F. at 9:30 a. m. to 98 F. at 10:15 a. m.

Fig. 4, Experiment 12.—The upper line is the carotid blood-pressure; the lower line is 50 mm. Hg pressure; *A* is the initial pressure, *B* the pressure after one hour, and *C* the pressure after two hours. This tracing shows the practical absence of change in the blood-pressure level when the animal is kept in water at body temperature.

TABLE 4.—RECORD OF EXPERIMENT 12

Time, A. M.	Temp. Water, F.	Temp. Cat, F.	Blood-Pressure mm. Hg	Respiration
10:20-10:25.....	100.4	98.6	136	36
10:30-10:32.....	100.4	98.6	150	36
11:00-11:05.....	100.4	100.4	144	40
11:30-11:35.....	98.6	99.5	144	40
12:20-12:25.....	100.4	100.0	136	40

It appears, therefore, that the fall in pressure was entirely due to the effect of heat on the organism.

DISCUSSION

Our chief interest centers about the fall in pressure which results from degrees of heat of not uncommon clinical occurrence. We know from daily observation that such amounts of hyperthermia do not cause serious organic damage. The hypotension of such conditions, therefore, must be evidence of a functional disturbance.

Since blood-pressure is determined by the energy of the heart as measured by the output per minute against resistance, and on the other hand by peripheral resistance, it is evident that the low pressure might be due to a lessened output from the heart, or to a diminished peripheral resistance, or to both; and the lessened peripheral resistance in turn might result from relaxation of the smooth muscles of the arterioles due

to the heat, or to fatigue of the nerve-cells in control of the vascular mechanism. The factors affecting peripheral resistance may be considered first.

It has long been held by clinicians that heat has a marked relaxing effect on smooth muscles, but they have consciously or unconsciously thought of this effect in relation to muscle which is undergoing abnormal tonic or clonic contractions. We know of no clinical evidence which may be presented to show that heat has the same effect on smooth muscle in a state of normal tonicity. DeZilwa[5] and Stewart[6] have shown experimentally that there is a gradual relaxation of the tone of smooth muscle as the temperature is raised from 10 to 40 C. (from 50 to 104 F.), but that temperatures over 40 C. cause a shortening again. In their experiments the amount of change occurring between 38.5 and 40 C. (101.3 and 104 F.) is very small. If we seek to explain our results as examples of the decrease of tonicity of smooth muscle in response to heat, we should expect to find that the pressure fell as the temperature rose to 40 C. (104 F.), and that the pressure rose thereafter with increasing temperature. Examination of our tables will show that just the opposite occurred. Therefore, we cannot logically attribute the hypotension to this factor.

In considering the other possible factor that might diminish peripheral resistance, we have to take into account the conditions of activities of nerve-cells. It is highly characteristic of nervous activity that periodic rest is needed to restore normal function. The observations of Hodge[7] showed that in nerve-cells definite physiological changes develop during the day and are recovered from during the rest at night. "Continuous loss of sleep is far more rapidly fatal than starvation, and the final changes, especially in the nervous system, are very marked" (Donaldson[8]). Howell has shown that sleep is accompanied by a diminution of vascular tone, that is, a lessened activity of the vasomotor center. Since, therefore, there is this alternation of fatigue and recovery through long periods during the twenty-four hours, and since the absence of the resting period is known to be harmful to the nerve-cells, the necessity for repair in these cells is obvious.

Studies of the effect of temperature on chemical processes have shown that there is approximately an increase of 10 per cent. in the speed of reaction with every increase of 1 degree centigrade. The metabolic processes occurring in nerve-cells are chemical in character, and a number of observations are on record to show how markedly the speed

5. DeZilwa: Quoted by Grützner, Ergebn. d. Physiol., 1904, ii, 12.
6. Stewart, C. C.: The Cat's Bladder, Am. Jour. Physiol., 1900, iv, 185.
7. Hodge: Quoted by Donaldson, The Growth of the Brain, London, 1895, p. 317.
8. Donaldson, H. H.: The Growth of the Brain, London, p. 309.

of nervous activity is augmented by increased temperature. Thus Helmholtz[9] found the velocity of the nerve impulse in the frog at 0 C. (32 F.) to be 2.7 meters per second; at 20 C. (68 F.) 27 meters per second. Von Miram[9] found the rate for the frog 0.24 meters per second at 20 C., and 65 meters at 35 C. (95 F.).

Since the evidence from activity and rest indicates that during the active period the catabolic changes are more rapid than the anabolic, it is highly probable that similarly increased heat has a greater effect on the catabolism of the neurons than on the anabolism. The increased speed of the catabolic processes which heat would induce might, therefore, result in increased tone of the vasomotor center in the early stages, and that period might be followed by a later period of diminished tone because of approaching exhaustion of the nerve-cells.

W. T. Porter[10] and his associates have brought forward evidence which indicates that the tone of the vasomotor center is not lowered by prolonged peripheral stimulation. Such stimulation, however, becomes widely dispersed through the nervous system and only in part affects the vasomotor center. Heat, one the other hand, would have as much effect on one cell as on another, and the fact that increased heat is accompanied by diminution of the tonic innervation of skeletal muscle indicates the possibility that a similar condition prevails in the vasomotor center.

In two of our experiments we tested the reflex response of the vasomotor mechanism after we had obtained large falls in blood-pressure. In every case the normal response occurred, but this evidence does not permit us to draw conclusions as to the tonus of the vasomotor cells, for Porter[11] has shown that the condition of the vasomotor center does not determine the presence or absence of the vasomotor reflex, and that consequently testing the reflex gives no information about the state of tonus of the center.

There still remains to be considered the effect of the heart rate on the lessened blood-pressure observed during continued hyperthermia. Experiments[12] have shown that the faster the heart beats the shorter the diastole, but within wide limits the abbreviation of the diastole occurs only in that part of it which has been designated diastasis; that is, the period of repose; and does not affect the period of relaxation or filling. If the heart is supplied with blood, a more rapid beat within the period

9. Helmholtz and Von Miram: Quoted by Snyder, Am. Jour. Physiol., 1908, xxii, 179.

10. Porter, W. T., Marks, H. K., and Swift, J. B.: The Relation of Afferent Impulses to Fatigue of the Vasomotor Center, Am. Jour. Physiol., 1907, xx, 444.

11. Porter, W. T.: Relation of Afferent Impulses to the Vasomotor Center, Am. Jour. Physiol., 1910, xxvii, 276.

12. Henderson, Y.: A Principle Underlying the Normal Variations in the Volume of the Blood-Stream, Etc., Am. Jour. Physiol., 1909, xxiii, 353.

of diastasis results in a greater output. Only when the beat becomes so rapid as to encroach on the period of filling does the output fail to vary with the rate of beat. The observations, reported in this paper, of increased blood-pressure in the early stages of hyperthermia and lessened blood-pressure after the temperature rose higher, are quite in harmony with the greater output from the heart that would result so long as the rapid beat encroached only on the period of diastasis, and with the lessened output that would result as the still more rapid beat would cause an encroachment on the period of relaxation or filling.

This review of evidence indicates that the heat probably does not cause sufficient local relaxation of smooth muscle in the arterioles to account for the low pressure. As between a central effect of the heat on the tonus of the vasomotor center and a peripheral effect on the heart, our observations thus far do not permit us to pass judgment. Quite conceivably both factors are involved in the production of effects which we have here reported, and as the foregoing discussion has shown, heat would operate in the same direction with both these factors to cause first a rise and later a fall of arterial pressure. For the present, therefore, we must leave open the explanation of our results and merely report the facts of observation.

CONCLUSIONS

1. In lower animals degrees of hyperthermia not greater than those encountered in infections are sufficient to cause marked hypotension.

2. The increased body temperature of infection is a potent factor in the production of the lowered blood-pressure which occurs in such conditions.

3. The hyperthermia may be the entire cause of such hypotension.

We are grateful to Dr. W. B. Cannon for having suggested the possible effect of heat on blood-pressure to us and for having aided us with many of the technical difficulties.

329 Beacon Street—374 Marlborough Street.

PAROXYSMAL AURICULAR FIBRILLATION*

G. CANBY ROBINSON, M.D.
ST. LOUIS, MO.

The abnormalities of the cardiac mechanism which are responsible for various forms of cardiac arhythmia and disturbances in cardiac rate are now well understood, and have been especially well demonstrated by means of the string galvanometer. The analysis of disturbances of the cardiac mechanism, although of ever increasing diagnostic and therapeutic importance, is a field for original investigation which is fast losing its fertility. On the other hand, large problems lie before us in determining the essential causes of these cardiac disturbances. The progress of the treatment of cardiac disorders will be accelerated by the discovery of the now unknown changes, either within the heart or outside of it, which are responsible for these abnormalities of the cardiac mechanism. This is especially true of the large group of disorders characterized by disturbances in the generation of stimuli of the heart beat. These disturbances may predominate either in the auricles or in the ventricles, and may result in the occurrence of premature ectopic beats (extrasystoles), tachycardia of ectopic origin, the so-called auricular flutter or auricular fibrillation. These disturbances may occur in hearts already the site of anatomical lesions, but they may also occur in hearts which give no signs of such lesions.

It is with the hope that some light may be thrown on this question of the essential cause of disturbed excitability or stimulus formation that the following case of paroxysmal auricular fibrillation is reported.

CASE REPORT

History.—I. G., Hospital No. 601, a jeweler, aged 50, born in Russia, was admitted to the hospital Feb. 23, 1912, complaining of cardiac palpitation and weakness. He knows of no cases of cardiac disease or rheumatism in his family, and the family history is otherwise negative. The patient had been a generally healthy man, but always "high strung" and rather nervous. He thought that he had neither scarlet fever nor diphtheria in childhood, but since the age of twelve he had frequent attacks of tonsillitis, and at the age of 15 typhoid fever. When 18 years old he had an apparently rather mild attack of gonorrhea, but there never had been any other venereal affection. His wife is living and well, and they have several healthy children. He had considerable distress after eating for many years, and had always taken large amounts of food, especially bread, and much tea. He used both alcohol and tobacco moderately and had

* Submitted for publication Sept. 24, 1913.

* From the Hospital of the Rockefeller Institute for Medical Research, New York.

never done laborious work. He never had rheumatism, but there was pain without swelling or redness in the right shoulder, beginning about eleven years ago and lasting fifteen months. He continued his occupation as a jeweler with very little interruption up to a few weeks before admission to the hospital.

The present illness began twelve years ago, in 1900, when the patient felt undue fatigue after exertion, and on the following day he had his first attack of cardiac palpitation and weakness, much like that for which he came to the hospital. He was in bed for three days at this time. He then had no such symptoms for about eighteen months, when the second attack occurred, and since then he has had similar attacks, usually lasting only a few hours, every four to six months. Occasionally they have lasted as long as three days. The patient thinks that the gastric disturbance, noticed especially after eating, had something to do with causing the attacks of palpitation. About eight weeks before admission the patient had the first of the series of attacks which brought him to the hospital. The first attack began Dec. 22, 1911, in the usual way, with a boring feeling in the epigastrium, a thumping feeling now and then in the heart, and finally a sudden onset of cardiac palpitation. There was also a feeling of pressure about the chest, and choking, headache and weakness. This attack lasted twenty hours and stopped suddenly. During the next three weeks the patient had two or three attacks of lesser severity, and then, Jan. 13, 1912, an attack began which lasted three and a half days. This was followed by four days when the patient felt quite well, at the end of which time the symptoms returned and continued for seven days. Then he was free from symptoms for ten days, when another attack, lasting a few days, occurred. Finally, after the patient had been feeling well for nearly two weeks, the final attack before admission began, and this time in an unusual way, a feeling of palpitation coming and going for a day or so, and then settling down on February 22, the day before admission, to what seemed to the patient a severe attack. Besides the symptoms that have been mentioned he had moderate pain in the left arm and in the back of the neck. He was able to walk into the hospital, but with some difficulty.

Physical Examination.—Physical examination showed the patient to be a rather poorly nourished, small man, who looked at least ten years younger than his age. He was lying quietly and apparently comfortably in bed, but appeared somewhat nervous. He was alert and highly intelligent. The eyes showed normal pupillary reactions to light and accommodation. The fundi were apparently normal except for slight tortuousness to the arteries. The tonsils were not enlarged. There was slight venous pulsation in the neck. The thyroid was not palpable. The lungs yielded normal physical signs throughout. There were no abnormal pulsations seen over the thorax, while there was a general heave in the region of the left nipple. The precordium was not prominent. The apex beat was in the fifth space, 10 cm. to the left of the midsternal line. The outline of cardiac dulness extended 4.0 cm. to the right of the midsternal line in the fourth space, and 11.5 cm. to the left of the fifth space. The heart sounds were clear throughout, no murmurs being heard. The second pulmonic was slightly accentuated, and had a liquid quality. The radial pulse was distinctly irregular, and had a rate of about 75 per minute. Many of the pulsations were well sustained, but they were irregular in both force and rhythm. The actual heart-rate as counted by auscultation over the heart was 126 per minute. The arteries were distinctly palpable in the arm, at the wrist and especially at the elbow. The temporal arteries were also indefinitely palpable. The abdomen was normal. Neither liver nor spleen was palpable. There was no edema of the extremities. Blood, hemoglobin 95 per cent. (Sahli) corrected reading = 119 per cent. Wassermann reaction was negative. The urine showed neither albumin nor casts. Its specific gravity was 1.030. The amount was 650 c.c. in twenty-four hours. Electrocardiograms showed that auricular fibrillation was present (Fig. 1.)

Subsequent Course.—Digitalis was begun three days after admission, and in three days distinct slowing of the ventricles had occurred and the arhythmia was much less marked. March 1, at 10:30 a. m., the patient was bled from an arm vein and 40 c c. of blood was obtained. At 11 a. m. he noticed a sudden change in the feeling of the heart beat, and declared that his attack was over. The examination revealed that the patient's heart was beating regularly, at first at a rate of 108, and in a few hours at about 90. The outline of relative cardiac dulness extended 4 cm. to the right in the fourth space, and 10 cm. to the left in the fifth space. The heart sounds were clear and of good quality all over the precordium. There were no murmurs, but the second aortic sound was accentuated. The patient felt immediately distinctly more comfortable, and seemed to be entirely well except for slight weakness. Electrocardiograms showed that the normal sequential beat had returned (Fig. 2).

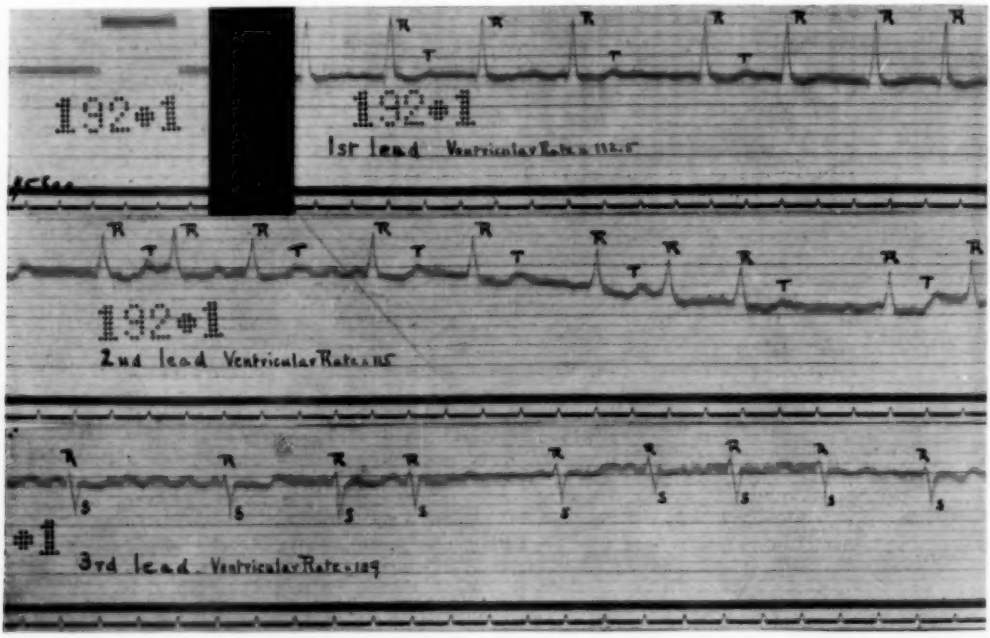


Fig. 1.—Curves 192. February 25. First, second and third leads, showing auricular fibrillation. Ventricular rate averages 112 beats per minute. The time marking is always in 1/5 seconds.

During the night of March 6, after the heart had been beating normally for about sixty hours, the patient felt an occasional jumping feeling in the epigastrium, which he said usually occurred before attacks, and which led him on this occasion to predict the return of cardiac palpitation. During the night it was noticed that cardiac arhythmia existed for a few minutes on several occasions.

The heart continued to beat regularly, however, until March 10, when the patient awoke at 1 a. m. with the sensation of weakness and felt that his heart was beating irregularly. When examined at 9:30 a. m. the typical arhythmia of auricular fibrillation was present, the diagnosis of which was confirmed by

electrocardiograms. This arhythmia continued until the afternoon of March 14, nearly five days. On this day the patient was restless, worried and especially uncomfortable in the morning, but in the afternoon the heart suddenly, practically instantly, became regular. The patient immediately felt much better, and except for muscular soreness and weakness was without symptoms. The cardiac examination gave results similar to those obtained during his first period of cardiac regularity. His condition remained excellent, and on March 22, after the heart had been regular for eight days, the patient was allowed to sit up out of bed for half an hour. March 23, a few minutes before 7 a. m., the pulse became suddenly irregular again. When seen a little later the patient was comfortable, and the heart, although completely irregular, was not beating very rapidly, and nearly all beats produced palpable radial pulsations. The heart sounds were clear. The arhythmia was still present on March 25, when the patient complained of feeling especially badly, with dizziness, weakness and a

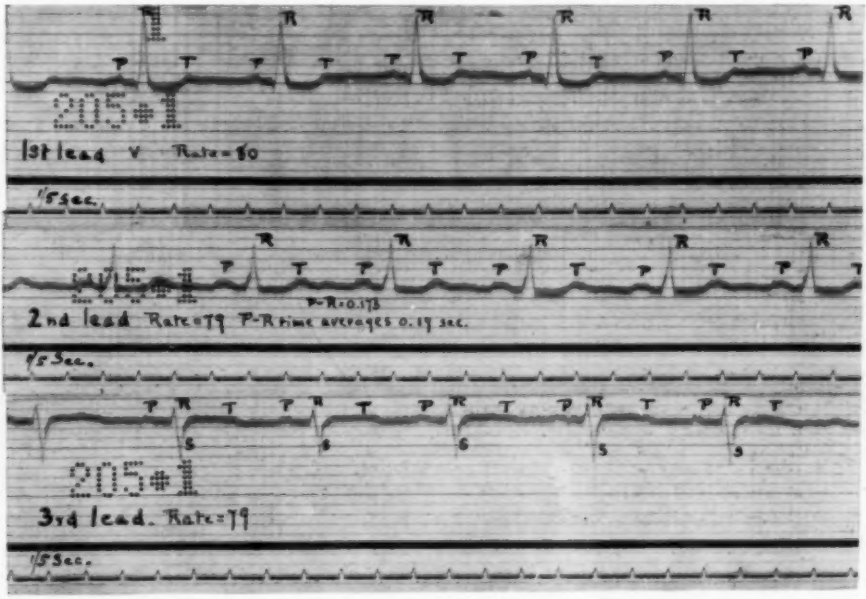


Fig. 2.—Curves 205. March 4. First, second and third leads obtained during the first period of cardiac regularity. The records show that the normal sequential beat is present. P.-R. time, second lead, average 0.17 second. Rate averages seventy-nine beats per minute.

feeling of helplessness. On March 26, at about midnight, the patient awoke to find that his pulse was regular and rapid, and when examined the next morning the heart was beating perfectly regularly as before. The outline of relative cardiac dulness extended 2 cm. to the right and 11.5 cm. to the left in the fourth space, and the heart sounds were as before. The cardiac rate was 80. Electrocardiograms again showed that the auricles were beating normally, and were once more properly coordinated with the ventricles. The patient seemed in excellent condition without symptoms, and the heart maintained a rate of about 72 for

several days. During the night of April 1, however, the patient had pain in the epigastrium and a feeling of suffocation, and on April 2, at 6 a. m., his pulse became suddenly irregular. When examined at 10 a. m., the heart was beating very irregularly, at a rate of about 140 per minute. Many beats failed to reach the wrist, and the radial pulse-rate was only about 100 per minute. The outline of relative cardiac dulness extended 3.5 cm. to the left in the third space, and 3.0 cm. in the fourth space, and 10.0 cm. to the left in the fourth space. The electrocardiograms were those of auricular fibrillation. From April 2 the cardiac arhythmia continued as long as the patient was under observation. The heart rate gradually slowed and became more nearly regular, but electrocardiograms showed that auricular fibrillation was still present when he was discharged on May 23, feeling quite comfortable and well. He had been walking about the ward for several days without symptoms. The arhythmia continued for several weeks after the patient left the hospital. It was subsequently learned that the patient died about two months later with symptoms suggesting acute dilatation.

DISCUSSION

During the time the patient was in the hospital he had three periods of nine, nine and six days, when the heart was beating regularly, between which auricular fibrillation occurred for five and four days. It was also present during the first six days and during the last fifty-one days in the hospital.

The change from one form of cardiac mechanism to another was usually sudden, but premonitory symptoms were sometimes noted by the patient before the onset of the arhythmia. These were, as have been described, a feeling of gastric distress and a thumping feeling in the cardiac region which was probably caused by the occurrence of premature ventricular contractions (extrasystoles). The occurrence of such beats was recorded by electrocardiograms several times during periods of fibrillation. The actual onset of auricular fibrillation occurred each time after the patient had been asleep, once apparently during sleep. On the first occasion the patient awoke at 1:00 a. m. with a sensation of weakness and felt that his heart was irregular, and the second and third occasions the arhythmia began suddenly at 7:00 a. m. and 6:00 a. m., respectively. Muscular exertion and psychic excitement, unless it was in dreams, played, therefore, no rôle in initiating auricular fibrillation. Gastric disturbance also did not seem to be a factor at such times. Thus, there was no evidence supporting the patient's statement concerning the influence of gastric distress on the cardiac rhythm, which may be a factor in the production of complete arhythmia, as v. Müller[1] has pointed out.

Auricular fibrillation changed to the normal sequential beat three times while the patient was in the hospital. The first change occurred at 11:00 a. m., half an hour after 40 c.c. of blood had been withdrawn from an arm-vein, the second during the afternoon following an uncomfortable and restless forenoon, while the third change occurred at midnight,

1. Müller, v.: The Nervous Affections of the Heart, THE ARCHIVES INT. MED., 1908, i, 1.

apparently during sleep. The changes were always sudden, and as the conditions under which they occurred were so different, no conclusions as to the possible cause of the changes can be drawn. The blood-letting was thought to have been a possible factor, but it was repeated on April 22, after fibrillation had been present three weeks, without any change in the cardiac mechanism. The patient stated that the return to the regular rhythm usually occurred when he was quiet.

The administration of digitalis may have been a factor in causing the disappearance of auricular fibrillation on two occasions. The drug was begun in the form of digipuratum tablets, 0.1 gm., three days after admission, five tablets the first day, decreasing one tablet each day. On February 29 the patient was nauseated after having had 1.5 gm. of digitalis, and the drug was discontinued at 3:30 p. m. The fibrillation ceased at 11:00 a. m. the next morning.

The drug was begun again in the same manner on March 11, two days after the onset of fibrillation, and continued for four days, when it was stopped after the sequential beat had returned. Small doses of the tincture, 0.2 c.c., three times a day, were given during this period of normal rhythm with the idea that it might assist in maintaining the regular beat, but on March 23 fibrillation again set in, and digipuratum was begun as before. This was continued for two days, and was discontinued after ten doses, as the patient complained of feeling dizzy and weak. Thirty-six hours after drug was discontinued, the normal beat again returned. The drug was withheld after the next onset of fibrillation had been present fifteen days, because it was felt that its administration probably had no effect in stopping the auricular fibrillation, and that the sequential beat would return spontaneously. As this did not occur, digipuratum was again administered. At this time and again later, it was given until nausea occurred, but produced no apparent effect on the auricular fibrillation. Digitalis was given primarily not with the idea of influencing the auricular activity, but because the rapid, irregular and insufficient ventricular activity present at the time of admission demanded it. In fact, as Mackenzie[2] and others have pointed out, digitalis may be apparently a factor determining the onset of auricular fibrillation. In our case fibrillation seemed prone to cease a day or two after the drug had been pushed to its physiological limit and discontinued. On one occasion fibrillation came on while small doses of the tincture were being taken.

The ventricular activity was favorably influenced by digitalis, and the rapid, irregular heart beat was reduced to a rate of about 80, and became almost regular, while fibrillation continued. During the periods of regular cardiac activity the electrocardiograms showed that there was no prolongation of the time of conduction from auricles to ventricles, the

2. Mackenzie: Digitalis, Heart, 1910-11, ii, 295.

P-R time in the second lead records averaging 0.17 seconds. This case does not lend support, therefore, to the suggestion put forward by Lewis[3] "that the slowing of the heart, when auricular fibrillation is present and digitalis is given, is due to an increase of a previously existing defect in the conduction to the ventricle of those impulses which are built up rapidly and irregularly in the auricle."

BLOOD-PRESSURE

The relation of changes in blood-pressure to changes in the cardiac mechanism was studied. The difficulty of determining the blood-pressure in cases of auricular fibrillation is evident, as the ventricular contractions, varying in force, produce variations in the blood-pressure from beat to beat. On account of the fact that exact estimations were impossible, the blood-pressure was not recorded at first, during auricular fibrillation, but later when the heart was beating more nearly regularly, the pressure was determined by the auscultatory method, at which the strongest beats produced sounds in the brachial artery and also at which practically all beats produced sounds. The average of these two readings was considered as the systolic blood-pressure. In a similar way two diastolic readings were taken and averaged. The blood-pressure readings are seen in the accompanying chart (Fig. 3). Although the blood-pressure was constantly high, marked changes took place, the systolic pressure varying from 125 to 215 mm. Hg. The diastolic pressure was more constant, but varied from 85 to 120 mm. Hg. In the second period of regular rhythm, from March 14 to 23, the blood-pressure rose gradually from 125 mm. Hg to 175, when fibrillation again set in. The blood-pressure did not fall with the onset of fibrillation, but twenty-four hours later it had fallen 25 mm. Hg, and remained relatively low until about thirty-six hours after the heart had become regular once more. Then the systolic pressure rose to 185 mm. Hg, remained high for three days, and then fell to 130 the day before fibrillation again set in. The blood-pressure was taken between 10:00 a. m. and 11:00 a. m. each day, and no observations were obtained immediately before and after a change in cardiac rhythm, so the day-to-day readings give no accurate indications as to changes in blood-pressure as a factor in causing changes in the cardiac mechanism. On the hypothesis that the high blood-pressure might be a factor in maintaining fibrillation, however, the patient inhaled 0.2 c.c. of amyl nitrite about three hours after the onset of fibrillation on March 23, at which time the systolic blood-pressure was 180 mm. Hg. He became nervous and dizzy, but except for the initial rapidity the heart beat did

3. Lewis: Auricular Fibrillation and Its Relation to Clinical Irregularities of the Heart, Heart, 1909-10, i, 306.

4. Lewis: Fibrillation of the Auricles; Its Effects Upon the Circulation, Jour. Exper. Med., 1912, xvi, 395.

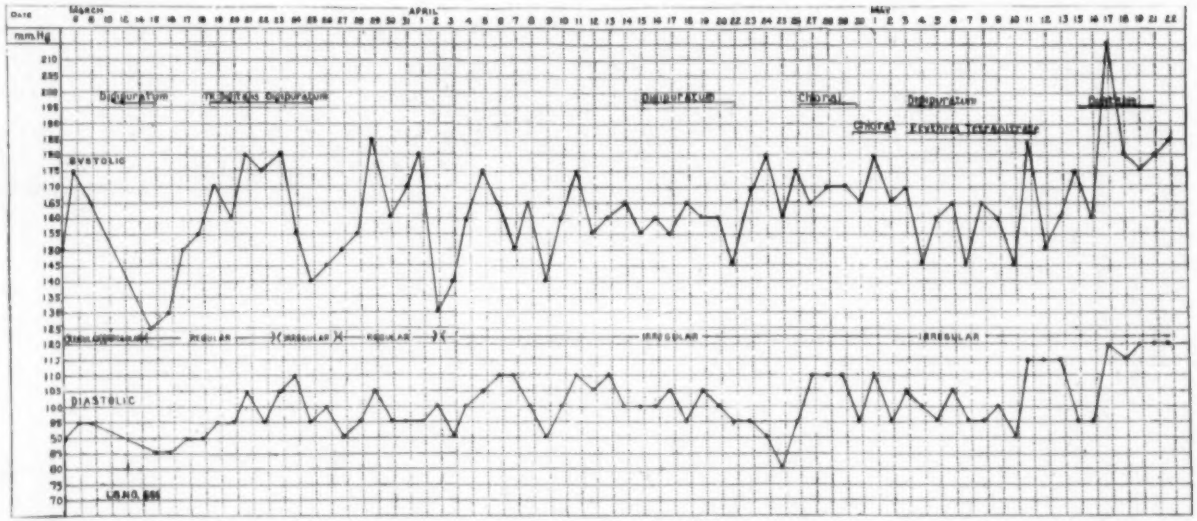


Fig. 3.—The systolic (upper line) and the diastolic (lower line) blood-pressure. The type of cardiac activity and the drug administration are also indicated.

not change, and the blood-pressure was apparently not lowered. On another occasion (April 22), three weeks after the onset of fibrillation, the systolic pressure fell during the operation of blood-letting to 115 mm. Hg without any change in the cardiac mechanism. Erythrol tetranitrate and chloral were also administered during fibrillation, but without affecting appreciably the blood-pressure or the cardiac mechanism. On the other hand, the systolic pressure reached 215 mm. Hg on one occasion during fibrillation without any change in the beat. It may be said, therefore, that no relationship between changes in blood-pressure and changes in the cardiac mechanism can be made out. The blood-pressure varied greatly, both during auricular fibrillation and during the normal rhythm, but the blood-pressure was maintained generally at a high lever, regardless of the form of cardiac activity present. These blood-pressure observations are in accord with those made by Lewis[4] on the experimental animal, in which he found that the onset of fibrillation was usually accompanied by a fall of blood-pressure, but the effect was inconstant. The circulation tended to accommodate itself to altered conditions, and to return to the same blood-pressure as that observed before the onset of fibrillation.

The effect of pressure on the neck over each vagus nerve was recorded by electrocardiograms several times, both during the normal rhythm and during fibrillation (Figs. 4-7). The abnormal susceptibility of the heart to mechanical stimulation of the vagi was striking. The length of time pressure was made over each nerve and the length of the responding pause may be tabulated as follows:

Auricular Fibrillation (Figs. 3 and 4)

	Pressure Over Right Vagus Seconds		Ventricular Stoppage Seconds	Pressure Over Left Vagus Seconds		Ventricular Stoppage Seconds
	3.0		3.3	3.4		4.8
	3.4		4.0	3.1		4.0
	2.8		4.0	3.6		4.4
	3.0		3.1	...		...
	3.0		3.4	...		...
Avearage.....	3.04		3.56	3.37		4.42
Proportion....	1	:	1.17	1	:	1.22

Normal Beat (Figs. 5 and 6)

	Pressure Over Right Vagus Seconds		Ventricular Stoppage Seconds	Pressure Over Left Vagus Seconds		Ventricular Stoppage Seconds
	1.4		3.2	2.0		3.6
	1.9		3.0	2.0		4.0
	1.9		3.0	2.0		1.9
Avearage.....	1.73		3.06	2.0		3.17
Proportion....	1	:	1.77	1	:	1.53

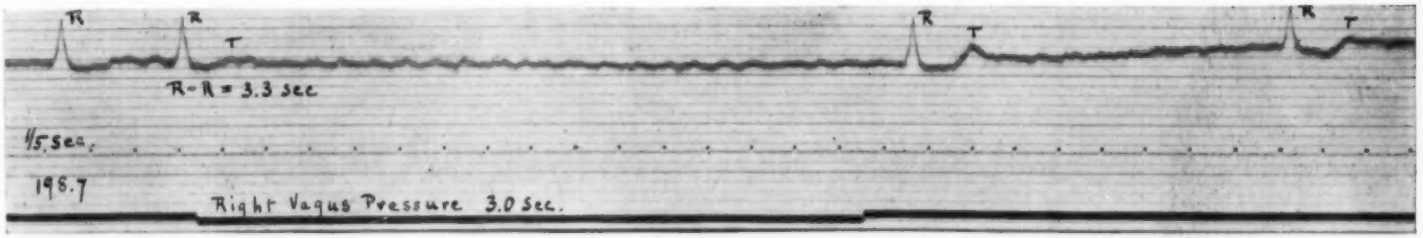


Fig. 4.—Curve 198.7. February 28. The effect of pressure for three secondsover the right vagus during auricular fibrillation.

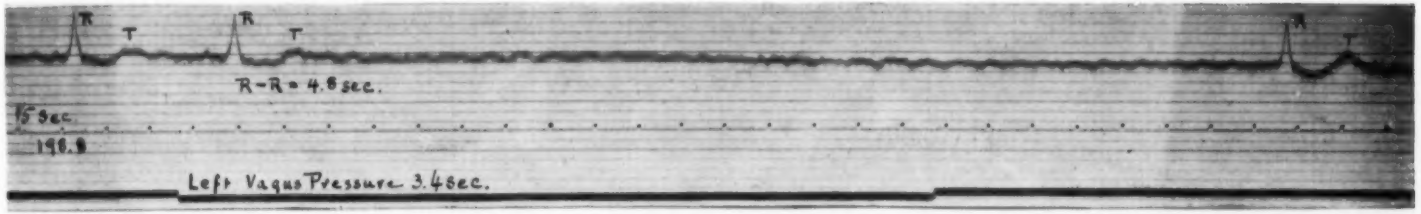


Fig. 5.—Curve 198.8. February 28. The effect of pressure for 3.4 seconds over the left vagus during auricular fibrillation.

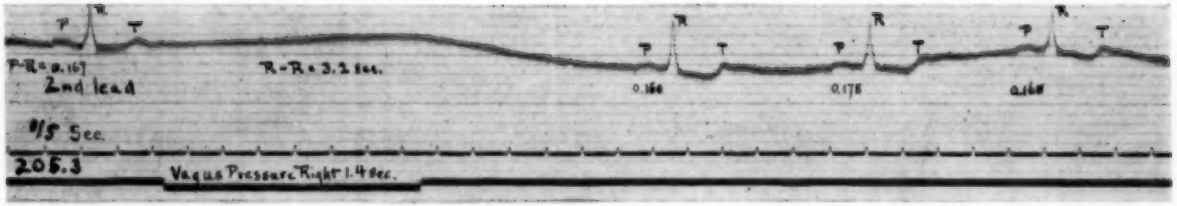


Fig. 6.—Curve 205.3. March 4. The effect of pressure for 1.4 seconds over the right vagus on the normally beating heart.

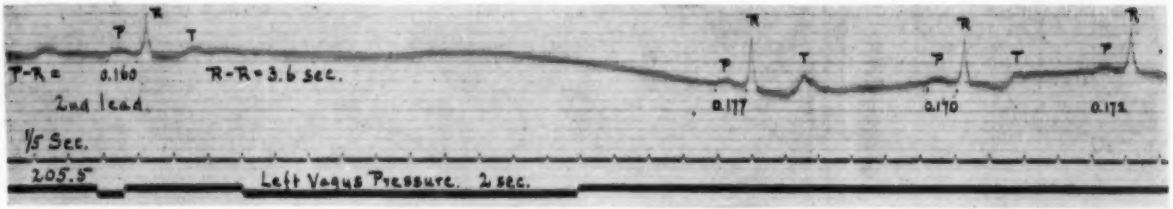


Fig. 7.—Curve 205.5. March 4. The effect of pressure for 2 seconds over the left vagus on the normally beating heart.

Thus it is seen that neither during auricular fibrillation nor during the normal beat can any definite difference be made out between the effects of right and left vagus pressure, such as can sometimes be demonstrated in man (Robinson and Draper[5]), and as can be almost constantly shown with faradic stimulation of the nerves in the dog (Cohn,[6] Robinson[7]). The ventricles were stopped for a longer time proportional to the length of pressure when the heart was beating normally than when auricular fibrillation was present, whether the left or the right nerve was pressed on.

The mechanism responsible for the ventricular pauses which followed vagus pressure during auricular fibrillation is apparently different from that which brings about the stoppage of the normally beating heart. The electrocardiograms indicate that the activity of the fibrillating auricles was unaffected by pressure over the vagi, while they show that the normally beating auricles were brought to rest by this procedure. The manner by which vagus pressure brings about the long cessation in the ventricular activity in cases of auricular fibrillation has been discussed by Robinson and Draper,[5] and it was concluded that the ventricular pauses are apparently due to the blocking of stimuli from the auricles. There was no evidence as to whether this striking effect depends on an increased susceptibility of the conducting fibers to vagus stimulation or whether it depends on the stimuli from the fibrillating auricles being of such a nature that only slight lowering of the conductivity succeeds in blocking them. The possibility must also be considered of some quantitative change in the auricular activity which is not to be detected in the electrocardiograms, but which makes it incapable of stimulating ventricular contractions.

When the heart was beating normally there is practically no evidence that pressure over either vagus nerve lowered the conductivity, as the P-R time was never prolonged beyond 0.21 second after the cardiac stoppage in any of the records. The marked effect on the rate of the whole heart is in sharp contrast to the effect on conduction. It may be, however, that this effect on rate is responsible for the lack of effect on conduction, for during the cardiac standstill the conductivity of the heart is freed from the influence of functional fatigue, and so has its normal capabilities at the end of the cardiac pause. The harmonizing of the effects of vagus pressure on the heart during auricular fibrillation with that observed when the heart was beating normally is difficult.

5. Robinson and Draper: I. Studies with the Electrocardiograph on the Action of the Vagus Nerve on the Human Heart, Jour. Exper. Med., 1911, xiv, 217; II. Studies with the Electrocardiograph on the Action of the Vagus Nerve on the Human Heart, Jour. Exper. Med., 1912, xv, 14.

6. Cohn: Jour. Exper. Med., 1912, xvi, 732.

7. Robinson: The Influence of the Vagus Nerves on the Faradized Auricles in the Dog's Heart, Jour. Exper. Med., 1913, xvii, 429.

The marked susceptibility of the heart to mechanical stimulation of the vagi may have been another expression of the cardiac abnormality which caused the auricular fibrillation. The hearts of patients with auricular fibrillation are often markedly affected by pressure over the vagi and an abnormal response to this procedure by a normally beating heart may possibly indicate that auricular fibrillation is more likely to occur in it than in a heart less affected by vagus pressure. It is difficult to say whether this abnormal response indicates a so-called heightened "vagus tone" or whether the heart itself is responsible for the marked response. A series of experiments were undertaken with the hope primarily that some new light might be thrown on the question of the relation of vagus activity and auricular fibrillation, but the results of the experiments[7] do not afford any positive evidence as to the rôle the vagi may play as a causative factor of auricular fibrillation in man. The dog's auricles may be thrown into fibrillation and tachycardia by direct faradization as readily after the vagi are cut as before, but vagus stimulation holds the auricles in their abnormal state of activity after it has been once set up by faradization.

The effect of atropin in our case was observed only during fibrillation, and after 0.9 mg. atropin had been given hypodermically the ventricular rate increased markedly from 84 to 158 beats per minute, recorded by an electrocardiogram taken forty minutes after the administration of atropin. The waves of fibrillation were not so distinct in the records obtained during the rapid ventricular rate as before. The great increase in ventricular rate following atropin indicates that the vagi were constantly strongly active at this time in controlling the ventricular rate. A desire not to disturb the normally beating heart prevented the use of atropin during the periods of regular rhythm.

A series of electrocardiograms were made directly from various points on the chest wall during the normal beat and during fibrillation. The technic described by Lewis[8] was followed, in the hope of gathering further evidence regarding the significance of the waves of auricular fibrillation in the electrocardiograms. The results were unsatisfactory, as neither the P waves nor the waves of auricular fibrillation were distinct.

When this patient is considered from an anatomical rather than from a physiological viewpoint, it is found that the heart is normal as regards its size, position and the character of the heart sounds. No valvular murmurs were ever heard, and although the second aortic sound was somewhat accentuated, the muscle sounds were of good quality. The outline of cardiac dulness was measured repeatedly, and no changes in

8. Lewis: The Origin of the Electric Oscillations and the Direction of Contraction of the Ventricle in Instances of Complete Irregularity of the Heart (Auricular Fibrillation), Quart. Jour. Med., 1911, v, 11.

size as those observed by Lewis and Schleiter[9] could be made out, as an accompaniment of the changes in mechanism. A slight increase in the size of the heart between the time of admission and of discharge was noted, however, the total width increasing 1 cm. during this time.

The constantly high blood-pressure and the palpable arteries in the arms and temporal regions led to the diagnosis of arterial sclerosis. The urine gave no evidence of diseased kidneys, as the usual tests were repeatedly negative except for several hyaline casts on two occasions. The functional activity of the kidneys was tested May 7 by the technic of Rowntree and Geraghty,[10] 6 mg. of phenolsulphonephthalein being injected intramuscularly. The first appearance of the drug in the urine was not definitely determined, but was estimated at about ten minutes. During the first hour 36.8 per cent. and during the second hour 21.9 per cent., or a total for two hours of 58.7 per cent. of the drug was excreted. Thus the functional activity of the kidneys was but slightly below the normal limits of excretion (40-60 per cent. the first hour, and 60-85 per cent. in two hours). The sole definite anatomical lesion, therefore, is apparently arterial sclerosis.

Arterial sclerosis is frequently a prominent characteristic of cases of auricular fibrillation. Thus Lea[11] found it present in 57.1 per cent. of the non-rheumatic cases in his series. No definite change in the cardiac muscle has been described in these cases with sufficient frequency to allow any conclusions to be drawn as to an anatomical basis for auricular fibrillation, and the post mortem examinations of some of the hearts in which fibrillation had been present reveal hearts which are anatomically sound (Gossage and Hicks[12]). Lewis[3] believes that it is changes in the walls of the auricles themselves which probably cause fibrillation, and he has expressed the opinion that anemia of the auricular walls may be a factor in setting up fibrillation. Changes in the blood-supply of the auricular walls may well have been an important factor in causing the attacks of fibrillation in our patient. The evident arterial sclerosis and the high but markedly changing blood-pressure might readily have caused abnormalities in the cardiac blood-supply, which not only rendered the auricles more prone to fibrillation and abnormally susceptible to vagus stimulation, but also furnished the immediate cause of an attack of auricular fibrillation by a temporary diminution of the cardiac circu-

9. Lewis and Schleiter: The Relation of Regular Tachycardias of Auricular Origin to Auricular Fibrillation, Heart, 1912, iii, 173.

10. Rowntree and Geraghty: An Experimental and Clinical Study of the Functional Activity of the Kidneys by Means of Phenolsulphonephthalein, Jour. Pharm. and Exper. Ther., 1909-10, i, 579.

11. Lea: Some Points in Relation to the Etiogoly of Auricular Fibrillation, Quart. Jour. Med., 1911, iv, 423.

12. Gossage and Hicks: On Auricular Fibrillation, Quart. Jour. Med., 1913, vi, 435.

lation. There is very little in support of this hypothesis, but arterial sclerosis and high blood-pressure have been prominent in several cases of transitory auricular fibrillation which have been observed. Thus Hornung[13] reports three cases in which these abnormalities were prominent features, and arterial sclerosis was also mentioned in two of Fox's[14] six cases. The case reported by Lewis and Schleiter, although only 28 years old, had somewhat thickened arteries, but on the one occasion recorded the blood-pressure was low.

The paroxysms of auricular fibrillation may occur over a period of many years, and our patient had them apparently for twelve years. One of Hornung's cases suffered with attacks of cardiac arhythmia for five years and another for at least ten, while one of the cases reported by Fox, the case originally observed by Cushing and Edmunds, had attacks for over twenty years. These cases, therefore, do not always deserve the bad prognosis which is usually accorded to auricular fibrillation. They may have anatomically normal hearts and be closely allied to cases of paroxysmal tachycardia, as Lewis and Schleiter point out. The prognosis may not necessarily be more grave than in tachycardia. However, the auricular fibrillation tends to become permanent, as Fox's cases well illustrate, and this is what happened in our case, as fibrillation was present during the last two months that he was in the hospital, and continued until his death about two months after his discharge. There is, therefore, apparently a group of cases which may be termed paroxysmal auricular fibrillation, in which arterial sclerosis may be an underlying cause of the abnormal cardiac activity, and in which attacks of fibrillation may recur over a period of many years in patients with anatomically normal hearts, without great impairment to the general health.

SUMMARY

A case is reported in which transient attacks of auricular fibrillation were observed and recorded by electrocardiograms. These attacks had apparently occurred over a period of twelve years, and auricular fibrillation became permanent while under observation. No definite cause could be discovered as initiating the attacks. No definite relation could be established between changes in blood-pressure, which was always abnormally high and variable, and the changes in the cardiac mechanism. The heart was strikingly susceptible to pressure over each vagus nerve, both during fibrillation and during the normal cardiac activity. This abnormality may be another expression of the changes causing auricular fibrillation. Electrocardiographic records of these vagus effects were obtained.

13. Hornung: Über atypische Tachykardische Paroxysmen, Deutsch. Arch. f. klin. Med., 1907, xci, 469.

14. Fox: The Clinical Significance of Transitory Delirium Cordis, Am. Jour. Med. Sc., 1910, cxl, 815.

The heart of the patient gave no signs of an anatomical lesion, but arterial sclerosis, a frequent accompaniment of auricular fibrillation, was present. The presence of this lesion and the high, inconstant blood-pressure suggest the possibility that changes in the cardiac blood-supply may have been the underlying causative factor in this case. Other cases showing attacks of auricular fibrillation occurring over a period of years have been reported in which high blood-pressure and arterial sclerosis were prominent features. These cases seem closely allied to paroxysmal tachycardia, and although the auricular fibrillation tends to become permanent, the prognosis for them is not necessarily so grave as that which is given in ordinary cases of auricular fibrillation with outspoken cardiac lesions.

1806 Locust Street.

THE MUTUAL ACTION OF CERTAIN DIGESTIVE FERMENTS *

J. H. LONG, Sc.D., AND G. W. MUHLEMAN, M.S.
CHICAGO

In the earliest days of the investigation of some of the important soluble ferments, or enzymes, the question of their behavior toward each other came up for study. In the human body we have to consider the action of the salivary, the gastric, the pancreatic and the intestinal ferments, and the behavior of each one of these may be modified not only by the reaction of the medium in which it acts, but also by the presence of others. Since the activity of the salivary diastase is not confined to the mouth, but is continued to the stomach, the effect of the hydrochloric acid and pepsin present has naturally been the subject of frequent studies. Practically, the behavior of the acid alone has real importance, since the ptyalin is so sensitive to the acid that even minute traces are sufficient to render it inert. That pepsin, also, may have an effect on ptyalin, or ptyalin on pepsin, are academic questions of little practical importance, and of no importance at all in the present inquiry.

Much more importance attaches to the inquiry, What is the action of the gastric juice, that is, of pepsin plus hydrochloric acid, on the group of ferments furnished by the pancreatic secretion? In the normal conditions obtaining in the animal body, and especially in the human body, it is evident that there is no interference of the one with the other. Passing down into the intestine remains of the gastric secretion, with acid reaction, become speedily neutralized and brought into a condition in which pepsin, if any of it remains, cannot act on either proteins or ferments. The question, however, becomes a very different one when we seek to determine the action of the pepsin, or the whole gastric juice, on artificial pancreas products given by the mouth, and which therefore must pass through the stomach.

This is the question which comes up in studying the behavior of the commercial product known as pancreatin, and we are permitted to ask about the action of these pancreas ferments, on the one hand, on pepsin, and, on the other hand, of the action of pepsin on them. The question is an old one and has been in the literature many years. We find that in his classic researches on the preparation and properties of trypsin Kühne, in the seventies of the last century, touched on it, and many subsequent investigators have dealt with some phase of it.

* Submitted for publication Jan. 5, 1914.

In view of the wide-spread use of pancreatin the inquiry has no little practical importance, and it comes to us now through questions raised in the investigations of the Council on Pharmacy and Chemistry on pancreas preparations which are supposed to resist the action of the gastric juice and exhibit their characteristic effects after passing through the stomach.

That "pancreatin" is much weakened in its action by contact with pepsin is often affirmed and perhaps generally believed, if one can judge by the somewhat vague statement of the Pharmacopeia, Eighth Revision: "The digestive power of pancreatin is injured by contact with pepsin in solution." Not only is this statement vague, but also it may not be altogether in accord with facts. The vagueness, however, is in a measure associated with the vagueness in the definition of pancreatin itself. Under the name "pancreatin" we understand a product obtained in some manner from the pancreas of the hog or ox, but as to the method of preparation or the ferment content of the article there is not much agreement. It is usually assumed that all the pancreas enzymes are present, but in the method of testing official in our Pharmacopeia only one of them is practically recognized and the content of this is assumed to be small. As to whether the determined value found for the amylopsin is to be taken as a measure of the content of the other ferments, or not, nothing is said.

What is called pancreatin is often not much more than fat-free ground pancreas. Perhaps this is usually the case, but in the older view, and that still held by many writers, pancreatin should be obtained by some process of *extraction* from the pancreas, and precipitation or concentration of the extract. The question of expense seems to prevent the manufacture in this way, as a rule, and there would be little or no advantage in the matter of stability.

Pancreas powders, or extracts, are in general very low in the lipase enzyme, and in the laboratory as well as in the manufacturing scale it is difficult to secure a permanent product which has much fat-splitting power. In dealing with "pancreatins," or other commercial products of this class, we are practically concerned with their amylolytic or proteolytic power only. To what extent, if at all, are these ferment activities injured by contact with gastric juice or its pepsin-acid equivalent? And in turn, have these ferments any action on pepsin?

These are the questions we have attempted to answer through a series of experiments.

1. THE ACTION OF HYDROCHLORIC ACID AND PEPSIN ON AMYLOPSIN

This is the statement of a problem which is more ideal than practically workable, since neither the amylopsin nor the pepsin is obtainable

in a pure or nearly pure condition. Both of the ferments, as we obtain them commercially, or as we prepare them in the laboratory, are associated with relatively large amounts of matter which, while inert as far as ferment action is concerned, may be very important in modifying ferment action. This is true of the phosphate and other salt content of the ferment products.

For the purposes of this inquiry the question is asked what is the behavior of the best obtainable laboratory or commercial products toward each other? and that is the problem to be discussed.

It requires but a brief study to show that the starch-converting ferment from the pancreas, as well as that from malt, is very sensitive toward acids.

Before touching the question of the pepsin behavior, it is therefore necessary to fix the limits of the acid action, as this, rather than pepsin, may be the main factor in the case. The Pharmacopeial statement is misleading in this respect. Various statements are found in the literature concerning the acid concentration which stops the amylolytic action of the ferments,[1] and these statements are often in conflict because the experiments on which they were based were doubtless made with products of very different degrees of purity, especially in respect to salt content. The phosphates of sodium, potassium, calcium and magnesium which are associated to some extent with most of these ferment products are important factors in modifying the action of added hydrochloric acid, and it must not be forgotten that the binding power of protein for acid is another factor.

To eliminate these factors as far as possible we have sought to find the behavior of the acid by using glycerol extracts of the ferment, as well as a considerable number of dry products.

These points, then, are to be considered under the general topic of pepsin-hydrochloric acid-amylopsin action:

A. Action of dilute acid on glycerol extracts and dry products from the pancreas.

B. Possible action of pepsin, alone, on these products.

C. Action of pepsin and acid on the same products.

The discussion of these problems must be preceded by an explanation of the methods of investigation employed, which, for the present purpose, need be but brief.

---

1. See, for example, the quotations in the work of Green, The Soluble Ferments and Fermentation, and the work of Oppenheim, Die Fermente und ihre Wirkungen; also Effront, Die Diastasen.

A. ACTION OF ACID ON GLYCEROL EXTRACTS

General Conditions

Glycerol extracts of the pancreas have been used by many observers, and in our own laboratory for years. The activity of such extracts and the best conditions for preparation have been most clearly pointed out by Vernon in a series of papers which have gone pretty fully into this phase of the subject.[2]

In our work the pancreas extracts were made by mincing the organs very fine and macerating through about three weeks with twice their weight of pure glycerol. The mass, thick at first, grew much thinner in time from the evident partial digestion. Finally the liquid was separated pretty thoroughly from the suspended particles by straining and by centrifugalization in a powerful machine capable of taking eight tubes of 100 c.c. each at one operation. In this way we secured about 1,600 c.c. of a cloudy liquid which deposited but little on standing. This was extremely active in amylopsin and trypsin, although prepared with careful exclusion from contact with any portions of the intestinal mucosa. Even without the addition of salt the liquid was markedly active on starch, but the addition of the salt brought an increase in the activity. In all our experiments aqueous dilutions of this extract were used, in some cases a 1:10 dilution, and in other experiments a 1:50 dilution, filtered.

Vernon and others who have worked with such extracts state that they retain their activity through months. With water solutions, however, the case is quite different, and in a 1:50 dilution, or even in a 1:10 dilution there may be a very marked loss of activity in from ten to fifteen hours, as when the solutions are allowed to stand over night. The loss of power is much increased by a slight elevation of temperature and at 40 C. may be very decided.

If, however, the dilutions are made with salt water in place of pure water, the stability is found to be much greater. These facts were illustrated by a large number of experiments made with some commercial pancreatin preparations, as well as with glycerol extracts, in an effort to find an explanation for the great variability in the relative strengths of the market preparations. It was found necessary, therefore, to use the water solutions of glycerol extracts as soon as possible after preparation in order to secure the maximum activity. This precaution is recommended by several manufacturing firms in methods given for testing their pancreatins.

---

2. Vernon, H. M.: These papers deal with tryptic as well as diastatic action. See Journal of Physiology, 1901, xxvi, 405; 1901, xxvii, 269, 316, and in other places.

The value of diastase preparations is practically determined by their action on starch paste. To secure definite and constant results much depends on the method employed in making the paste, and for details on this point we refer to the general scheme as followed in this laboratory and described by Johnson and one of us[3] in a recent publication.

Method of Observation

In following out the action of diastases on clear starch paste made up usually to the strength of 10 gm. of anhydrous starch to the liter, we employ the form of the iodin test worked out in this laboratory and reported by Johnson.[4] In the digestions, always at 40 C., we carry the reaction to the colorless end-point, when 5 drops of the digesting fluid are added to 5 c.c. of a dilute iodin solution, made by dissolving 1 gm. of iodin and 2 gm. of potassium iodid in 125 c.c. of water and diluting 2 c.c. of this solution to 1 liter, finally, for use.

When the dilute starch paste is added to this iodin solution the blue color comes out sharply. As digestion proceeds the blue changes to a purple tint, then a purplish red, a rather deep red, a light red or reddish, a yellowish shade and finally colorless, or at most the faint shade of the weak iodin solution. It may be advantageous to use even a weaker iodin to note the final tint, but usually we do not do this. When used with pancreatic preparations Sherman[5] objects to this method of following the reaction because of the appearance of the red shades. But it is just this appearance and the gradual changes which constitute the most desirable features of the iodin test. Its advantages have been pointed out by others.[6] The conversion of starch to sugar is far from being an immediate process, and whether the dextrins are definite and sharply definable bodies or not, we must admit that there are various stages in the change from the original starch to the final malt sugar. The purple color which succeeds the blue corresponds to the so-called amylodextrin of some authors, and its appearance is a valuable indication of the progress of the action. This stage is followed by that of other mixtures, not definite, of course, but all clearly suggesting the rapidity of the transformation. It is desirable to employ a method in which this transformation may be conveniently followed, and this is true of the iodin method as used by us. At the end we have just as much information as may be derived from the copper reduction tests, which we have often used for controls, and much additional information, besides, which may be lost by making the copper reduction tests in the presence of an excess of starch. In tech-

3. Long and Johnson: Jour. Am. Chem. Soc., 1913, xxxv, 896.
4. Johnson: Jour. Am. Chem. Soc., 1908, xxx, 798.
5. Sherman and Colleagues: Jour. Am. Chem. Soc., 1910, xxxii, 1081.
6. See Roberts: Proc. Royal Soc., 1881, xxxii, 145; Detmer: Ztschr. f. physiol. Chem., 1882, vii, 1.

nical inquiries as to the value of relatively strong diastase preparations the copper methods are undoubtedly the most satisfactory, but in a strictly physiological study, where the time element is important, certain advantages seem to rest with the iodin process.

With this general discussion of the preparation of the materials and the method of testing we are ready for a description of the experiments carried out to test the behavior of weak acids toward the starch-amylopsin reaction.

Like the other actions, that of acids on the starch-digesting ferment of the pancreas has been much discussed. It seems desirable to bring out certain conditions here which have not been clearly recognized, although they have been in part pointed out by previous writers.

Amylopsin is much more sensitive to the action of traces of acids than to alkalies, and the final effect is more or less dependent on the presence of salts, which may not only serve to stimulate the catalytic action of the ferment, but also act to depress the activity of the acid hydrogen in its paralyzing behavior. Attempts have been made to show that diastase absorbs gaseous hydrochloric acid through some other than a basic property, and that a large part of the gas may be removed by suction without having fully destroyed the ferment. Panzer[7] has published a lengthy investigation on this subject, but the weight of acid added to the ferment preparations was in all cases so large as to permit no conclusion concerning the behavior of the small acidities which really come into consideration where the physiological action is concerned. The same is true of the experiments where aqueous acid was used with gram or half-gram weights of the ferment. In one case 1 gm. of a certain diastase preparation and 0.06775 gm. of hydrochloric acid, made up to about 80 c.c., stood an hour and was then neutralized with sodium hydroxid and diluted to 100 c.c. Portions of this dilution were then allowed to act on starch paste, the iodin and sugar tests being made from time to time. The figures given show a greatly diminished diastatic conversion, as one might expect from the rather high acid concentration. In discussing this and other experiments, the author states that the small amount of salt formed in the neutralization cannot be in any way responsible for the modification of the diastatic action, since such small quantities are of no significance. This conclusion does not appear to be warranted by facts already referred to, which will be brought out below. The salt here is undoubtedly of very considerable importance.

In our tests we considered the behavior of those small weights of acid which have practical significance. Small definite volumes of the glycerol extracts, diluted, were mixed with known weights of acid, and

7. Panzer: Ztschr. f. physiol. Chem., 1912, lxxxii, 276.

the mixtures made to constant volumes. These were then incubated at 40 C. through a period of half an hour, usually, after which a constant volume of the 1 per cent. starch paste was added and the incubation continued. From time to time drops were taken from the incubating mixtures to test for the disappearance of the iodin-starch reaction which would show the conversion of the starch to sugar or forms of dextrin which do not react with iodin. The rapidity of this conversion is a measure of the activity of the amylopsin present. As the action of the acid becomes evident, the time required to convert all the starch to products not giving a color with iodin is lengthened.

Two conditions in the last steps of the reaction may be noticed. After the incubation of the ferment and acid mixture the starch may be added directly and a new incubation made, or, after the preliminary incubation, a little weak alkali may be added to neutralize the acid before mixing with the starch paste. In the first case the starch digestion takes place in a slightly acid medium, while in the second it is neutral. Both lines of observation were followed.

In all cases some salt was added to the amylopsin extracts, to bring them all up to the degree of maximum activity which may be secured in this. The importance of this in comparative tests has been frequently pointed out.

EXPERIMENT 1.—Ten c.c. of a glycerol extract of pancreas made by diluting the original thick extract with water in the proportion of 1 : 49. One hundred mg. of sodium chlorid added. Amounts of hydrochloric acid as shown below were added from dilute solution and the whole diluted to 50 c.c. for each test, carried out in 200 c.c. flasks. Incubated thirty minutes at 40 C. and added to each mixture 50 c.c. of 1 per cent. starch paste. Incubated again and withdrew, from time to time, 5 drops from each flask for iodin tests.

In a control flask no acid was used, and tests made with this at the end of three minutes showed complete absence of color, indicating total conversion of the starch. In five other flasks, with 1, 3, 5, 7 and 9 mg. of acid there was no perceptible conversion, as a strong iodin reaction was found in each case, even after an hour of incubation with the starch. In a parallel experiment the acid, after the preliminary incubation of thirty minutes, was neutralized with sodium bicarbonate. In the following incubation with starch paste the same results were obtained as before.

EXPERIMENT 2.—Ten c.c. of a 1 : 10 dilution of the glycerol extract were taken for each test and with 100 mg. of salt diluted to 50 c.c. Each test flask contains, then, in the 50 c.c. 1 c.c. of the original glycerol extract, and weights of acid as in Experiment 1. After thirty minutes incubation at 40 C. 50 c.c. of 1 per cent. starch paste was added to each flask and final incubation began. In the control flask, with no acid added, the starch reaction disappeared in three minutes. In a flask with 1 mg. of hydrochloric acid the starch reaction began to fade in less than six minutes, was weak in ten minutes, and absent in an hour. With stronger acid concentrations, 3 mg. and above, the starch reaction was always sharp, even after one hour.

EXPERIMENT 3.—Conditions same as in 2, but twice as much of the glycerol extract present. In the flask with 1 mg. of acid present the starch reaction disappeared in the same manner as before, but with the concentrations of 3, 5, 7 and 9 mg. of hydrochloric acid there was a perceptible change in the starch reaction in an hour. The blue color had given place to purple.

In this last experiment there is a suggestion that with larger initial amounts of the pancreas ferment present there is greater practical resistance to the acid. Some of the ferment seems to have escaped destruction and is able to bring about a slow conversion of starch. In the following experiment still more of the ferment was used.

EXPERIMENT 4.—For each test 5 c.c. portions of the original glycerol extract were diluted with water to 50 c.c., receiving at the same time weights of acid as given in Table 1 and 100 mg. of salt in each case. After the preliminary incubation of thirty minutes at 40 C., 50 c.c. of paste was added to each flask and the final incubation started. The results are shown in Table 1. Complete destruction of the starch, as shown by absence of iodin reaction, is indicated by +; when there is no conversion of starch this in indicated by —. Partial conversion is indicated by $\mp$, with this sign to indicate a progressive change $\rightarrow +$.

TABLE 1.—DIGESTION OF STARCH WITH PANCREAS EXTRACT PREVIOUSLY INCUBATED WITH HYDROCHLORIC ACID AND SALT. (EXPERIMENT 4)

	Time of Incubation, Minutes					
HCl Mg.	3	6	10	15	30	45
0	+	+	+	+	+	+
1	+	+	+	+	+	+
3	+	+	+	+	+	+
5	+	+	+	+	+	+
7	$\mp$	+	+	+	+	+
9	$\mp$	$\rightarrow +$	+	+	+	+
11	$\mp$	$\rightarrow +$	$\rightarrow +$	$\rightarrow +$	$\rightarrow +$	$\rightarrow +$

With this stronger concentration of enzyme, its destruction appears to be very much delayed. The results seem to suggest that the effect depends not only on the acid concentration, but on the enzyme concentration also. In other words, a given weight of acid will destroy or inhibit the action of a given amount of enzyme only. A weight combination between the two is suggested in this way, but there is another possibility which must be noticed, and that is that the increasing weight of inert protein substance added with the largest weights of ferment binds to some extent the acid and weakens its action.

The amount of protein present, however, is in no case sufficient to hold enough of the acid to effect a marked change in its concentration. Certain salts present may have a larger influence on the behavior, as will be pointed out below.

A moment's consideration will suggest that the absence of digestive power on the part of the ferment, as shown by the starch test made, may be explained in two ways: first, the ferment may be destroyed in the incubation process, or second, the ferment may not be destroyed, but its activity toward the starch may be hindered by the presence of the acid. It is well known that larger quantities of acid will stop conversion of the starch, and possibly with the smaller quantities here employed this may take place, without involving the actual destruction of the enzyme. Some

experiments with an active commercial pancreatic diastase throw light on this point. This product contains considerable chlorid, from the process of manufacture.

EXPERIMENT 5.—Pancreas powder, 500 mg. to 100 c.c. plus 3 mg. of hydrochloric acid. Incubated 50 c.c., *a*, through thirty minutes at 40 C., and allowed the other 50 c.c. *b*, to stand thirty minutes at room temperature. Then mixed small volumes with 50 c.c. of starch paste, 1 per cent., incubated again, and tested after the intervals as shown in Table 2. The degree of conversion is indicated as in 4.

EXPERIMENT 6.—Conditions same as in 5, but 6 mg. of hydrochloric acid was present in the 100 c.c. with the 500 mg. of pancreas powder (Table 3).

EXPERIMENT 7.—Conditions as in 5 and 6, but 12 mg. of hydrochloric acid present in the 100 c.c. for the preliminary incubation (Table 4.)

TABLE 2.—DIGESTION OF STARCH WITH PANCREAS EXTRACT PREVIOUSLY INCUBATED WITH 3 MG. HYDROCHLORIC ACID. (EXPERIMENT 5)

Ferment Solution	Time of Action on Starch, Minutes						
	a					*b*	
c.c.	5	10	20	40	60	5	10
4	—	→+	→+	+	+	+	+
6	—	→+	→+	+	+	+	+
8	—	+	+	+	+	+	+
10	—	+	+	+	+	+	+

TABLE 3.—DIGESTION OF STARCH WITH PANCREAS EXTRACT PREVIOUSLY INCUBATED WITH 6 MG. HYDROCHLORIC ACID. (EXPERIMENT 6)

Ferment Solution	Time of Action on Starch, Minutes							
	a					*b*		
c.c.	5	10	15	25	35	5	10	15
4	—	—	—	—	—	→+	+	+
6	—	—	—	—	—	+	+	+
8	—	—	—	—	—	+	+	+
10	—	—	—	—	—	+	+	+

TABLE 4.—DIGESTION OF STARCH WITH PANCREAS EXTRACT PREVIOUSLY INCUBATED WITH 12 MG. HYDROCHLORIC ACID. (EXPERIMENT 7)

Ferment Solution	Time of Action on Starch, Minutes							
	a					*b*		
c.c.	5	10	15	25	35	5	10	15
4	—	—	—	—	—	→+	+	+
6	—	—	—	—	—	+	+	+
8	—	—	—	—	—	+	+	+
10	—	—	—	—	—	+	+	+

With the smallest acid concentration, 3 mg. of hydrochloric acid to 100 c.c., it is seen that the preliminary incubation does not completely destroy the ferment action. It is somewhat delayed, however. Possibly, the action of the acid is much modified by the protein and salts present. But with 6 and 12 mg. of acid to the hundred cubic centimeters, with the 500 mg. of pancreas powder there is a very different picture. In the incubated portion there appears to be a complete destruction of the enzyme, since no change is found in the reaction with starch paste with

the largest amount of ferment solution or after prolonged action on the starch. In the portions not incubated, with the acidity the same, starch conversion goes on rapidly in all cases.

These experiments show that the amounts of acid used, which are sufficient to destroy the ferment in incubation, are not sufficient to impede the actual digestion.

The acid effect is shown in the incubation with the diastase, not in the behavior of the diastase with starch. Other experiments showed that starch conversion will go on in presence of much larger weights if acid, but it is evident that very small concentrations of acids destroy the ferment at the incubation temperature.

In this connection it may be asked what is the effect of the addition of alkali soon after the addition of acid to the ferment? In the experiments of Panzer, referred to, an attempt was made to answer this question, but the somewhat unfavorable conditions of the experiments made the answer uncertain. Some of our experiments on this point suggested a partial recovery of activity, by speedy addition of alkali. In mixtures incubated for some time the addition of alkali had but little effect, but when sodium bicarbonate was added soon after the acid, with no incubation, the result was somewhat different, as shown by these experiments.

EXPERIMENT 8.—Ten c.c. portions of a 1 : 10 glycerol extract of pancreas (1 c.c. of the original strong extract diluted to 10 c.c. with water) was mixed with 100 mg. of salt and acid as below and diluted to 50 c.c. No preliminary incubation. Each portion was then incubated with 50 c.c. of 1 per cent. starch paste for the time shown in Table 5.

TABLE 5.—DIGESTION OF STARCH WITH PANCREAS EXTRACT MIXED WITH SALT AND HYDROCHLORIC ACID. (EXPERIMENT 8)

	Time of Action on Starch, Minutes					
HCl Mg.	3	6	10	15	30	90
0	+	+	+	+	+	+
1	+	+	+	+	+	+
3	—	—	—	—	—	—
5	—	—	—	—	—	—
7	—	—	—	—	—	—
9	—	—	—	—	—	—

EXPERIMENT 9.—Conditions as in 8. But immediately after mixing with the acid and shaking, the equivalent weights of sodium bicarbonate were added. That is, the acid was neutralized as quickly as possible, so that its action on the ferment solution was for a brief time only. Then each portion was mixed with the starch paste and incubated as before (Table 6).

TABLE 6.—DIGESTION OF STARCH WITH PANCREAS EXTRACT, MIXED WITH SALT AND HYDROCHLORIC ACID, NEUTRALIZED WITH SODIUM BICARBONATE. (EXPERIMENT 9)

	Time of Action on Starch, Minutes					
HCl Mg.	3	6	10	15	30	60
0	+	+	+	+	+	+
1	+	+	+	+	+	+
3	→+	+	+	+	+	+
5	—	—	→+	→+	→+	→+
7	—	—	—	—	—	—
9	—	—	—	—	—	—

With the weights of acid here used there seems to be a practical destruction of the ferment in a minute or less, of action. The failure to convert the starch is not due to the presence of acid, since in Experiment 9 the acid is neutralized before ferment solution and paste are mixed. The reaction here is neutral. It will be noticed that with the smaller acid concentration there is some little effect by speedy addition of the bicarbonate, but it is not very marked. Unless protected by protein or salts the ferment is very rapidly injured or destroyed by traces of acid.

There appears, at first sight, to be a discrepancy between the last results and those of Experiment 7, in which 12 mg. of acid with ferment diluted to 100 c.c., but not incubated, left the diastatic activity still marked. In this case, however, the ferment was in the form of 500 mg. of a pancreas powder, and the effect of the protein and salts present would doubtless be of moment. The glycerol extracts do not contain much protein.

From all these experiments it appears that very low concentrations of acid destroy amylopsin. Experiment 8 shows that 3 mg. of the acid, not neutralized at once, destroys the diastase activity to such an extent that there is practically no starch conversion in ninety minutes. This is a strength of 0.006 per cent. in the ferment solution, before adding the starch. That is, 1.6 c.c. of 0.1 normal hydrochloric acid to 100 c.c. destroys even the beginning of starch conversion. Kendall and Sherman[8] have shown that about one-fourth of this stops the conversion to the sugar stage in a diastase-starch mixture. Our results are not very different from those found by Kjeldahl for the effects of dilute sulphuric acid on malt infusions. He found that 10 mg. of the acid for 100 c.c. of paste and infusion practically prevented the starch conversion.[9]

Langley and Eves[10] state that 0.005 per cent. of hydrochloric acid is destructive to the action of ptyalin, while one-third of this slows the reaction perceptibly, which accords pretty well with our experience.

But it must be recognized that the amount of the ferment, or the accompanying proteins, plays a very important part here, as shown by Experiment 4, in which a dilution of the same glycerol extract, but five times as strong as in 2, was employed. In that experiment, even after incubation, a marked diastatic activity was found in a solution with 18 mg. hydrochloric acid to 100 c.c. of ferment solution. This fact may reconcile the somewhat conflicting statements given by Langley and Eves[10] and by Chittenden and Ely[11] for the destructive effect of the acid.

8. Kendall and Sherman: Jour. Am. Chem. Soc., 1910, xxxii, 1091.
9. See Effront: Die Diastasen, 1900, p. 121.
10. Langley and Eves: Jour. Physiology, 1883, iv, 18.
11. Chittenden and Ely: Am. Chem. Jour., 1882, iv, 107.

Small amounts of acid are undoubtedly stimulative under certain conditions, and often by the formation of an alkali salt. Where relatively large amounts appear to have but slight action, it may be on account of the presence of much protein, which combines with the acid, or on account of salts like bicarbonates or phosphates, which in a sense neutralize it through liberation of a weaker acid of lower dissociation. It is certain that the ferment, not protected in some such manner, is extremely sensitive.

It is doubtless true that the hydrogen ion content of the solutions in question is the important factor in determining the action on the ferments. This has been shown for a number of enzymes and is probably the case here. In this connection the papers of Sörenson[12] deserve consideration. But in addition to the effect of the hydrogen ions there is also a neutral salt effect which is not so clearly understood and which complicates the general behavior.

B. THE ACTION OF PEPSIN ON AMYLOPSIN

We come now to the consideration of the behavior of pepsin alone toward the solutions of pancreatic diastase. It is commonly held that pepsin is without any action in neutral solutions or solutions of very low acidity, and this is doubtless true in respect to the behavior with coagulated egg albumin and other common protein substances. It does not *necessarily* follow that the same should hold for diastase or other enzymes. There appear to be no very definite statements on this point in the literature. Experiments reported by Wroblewski, Bednarski and Wojczynski appear[13] to lead to the conclusion that a pepsin solution in absence of acid is able to partially destroy malt diastase, and that when acid is present the pepsin increases the action of the latter. The diastatic activity is measured by copper suboxid reduced from Fehling solution, but the results of a series of tests are so irregular as to leave one in doubt as to their interpretation. We have carried out some tests with glycerol extracts of pancreas and with active commercial preparations from the gland. The general method of observation consisted in noting the rate and extent of action on standard starch paste of a series of pancreatic solutions, some of which were not mixed with pepsin solutions, while others received an addition of pepsin solution, in some cases boiled and in others not. In such experiments it must be kept in mind that incubation alone materially weakens the strength of amylopsin solutions. Observations must be made in sufficient number to measure and allow for this behavior. When precautions are taken to have the salt content

12. Sörenson: Biochem. Ztschr., 1909, xxi and later.

13. Wroblewski, Bednarski and Wojezynski: Beitr. z. chem. Physiol. u. Path., 1902, i, 229.

and other conditions the same it cannot be discovered that pepsin in neutral solution has any effect on the conversion of starch by amylopsin.

These necessary conditions are secured here by treating the diastase solution in parallel experiments by active pepsin and pepsin rendered inactive by heat. In tests made with the laboratory pancreas extracts and commercial products no definite effect could be proved.

C. THE ACTION OF PEPSIN PLUS HYDROCHLORIC ACID ON AMYLOPSIN

It has been shown above that even very weak acid has a destructive or inhibiting action on the pancreatic diastase, and that pepsin alone has little or no action on the same ferment. It remains to inquire what is the behavior of pepsin *plus* an acid of a strength below the destructive strength on the ferment. Will pepsin aided by such an extremely weak acid have any action in excess of that of pepsin alone? It is well known that as far as the action on egg albumin is concerned an acid of appreciable strength is required to bring about digestion, but a weaker acid may be sufficient to exert some effect when pepsin is present. If a weak acid retards the action of amylopsin to a certain degree, will the presence of pepsin retard it still more? This is one way to approach the question, and we have carried out a number of experiments in this direction, which are here explained.

EXPERIMENT 10.—Five portions of a glycerol extract of pancreas were made up to 100 c.c. with acid and pepsin as shown in Table 7. These mixtures were incubated through three hours at 40 C. and then 1 c.c. of each was incubated again with 25 c.c. of 1 per cent. starch paste. The results are shown in Table 7.

TABLE 7.—DIGESTION OF STARCH WITH PANCREAS EXTRACT, PREVIOUSLY INCUBATED WITH HYDROCHLORIC ACID AND PEPSIN. (EXPERIMENT 10)

Test No.	HCl Mg.	Pep. Mg.	1 Per Cent. Sarch, c.c.	Conversion
1	5	100	25	No conversion
2	5	50	25	42 minutes
3	5	25	25	27 minutes
4	5	—	25	20 minutes
5	—	—	25	11 minutes

The 5 mg. of acid in 100 c.c. equal 0.005 per cent., which amount of acid was found before to have little destructive effect on the amylopsin. It may delay the reaction to the colorless end-point, but does not kill the amylopsin. The long incubation is more important than the acid in this case. With increasing amounts of pepsin we have a check on the rapidity of the conversion, and with 100 mg. of pepsin present the reaction becomes so slow as to escape notice.

Another set of experiments was arranged in a different way as shown.

EXPERIMENT 11.—Ten c.c. portions of a 1 : 10 glyceral extract of pancreas were mixed with hydrochloric acid and pepsin to make 25 c.c. of solution as

shown in Table 8. These mixtures were incubated thirty minutes at 40 C. and then, after neutralization, incubated with 25 c.c. of 1 per cent. starch paste. The mixtures were tested by the iodin reaction at different intervals.

TABLE 8.—Digestion of Starch with Pancreas Extract, Previously Incubated (Thirty Minutes) with Hydrochloric Acid and Pepsin and Neutralized. (Experiment 11)

Test No.	HCl Mg.	Pepsin Mg.	Incubation with Starch and Result After Minutes 3	8	13	18	30
1	0.4	10	—	—	—	—	—
2	0.4	..	→+	+	+	+	+
3	0.8	10	—	—	—	—	—
4	0.8	..	—	—	→+	→+	→+
5	1.2	10	—	—	—	—	—
6	1.2	..	—	—	—	—	—

In the pepsin-containing mixtures the conversion of the starch is very much delayed, even with the weaker acid content. In absence of pepsin, with the lower acid concentrations, we have a distinct conversion of starch.

Experiment 12.—Same as 11, but with a preliminary incubation of ten minutes (Table 9).

TABLE 9.—Digestion of Starch with Pancreas Extract, Previously Incubated (Ten Minutes) with Hydrochloric Acid and Pepsin and Neutralized. (Experiment 12)

Test No.	HCl Mg.	Pepsin Mg.	Incubation with Starch and Result After Minutes 3	8	13	18	30
1	0.4	10	—	—	—	—	—
2	0.4	..	+	+	+	+	+
3	0.8	10	—	—	—	—	—
4	0.8	..	→+	→+	→+	→+	→+
5	1.2	10	—	—	—	—	—
6	1.2	..	—	—	—	→+	→+

When the mixtures with the pepsin were given more time it was found that a partial change of the starch followed, but slowly. There appears to be, therefore, some action of the pepsin and acid which the acid alone does not possess. In a later series of experiments the method was varied by adding boiled pepsin solution in the control. This introduced the same amount of protein. But the results were of the same order. With the active pepsin present in the preliminary incubation there was a delay later in the starch digestion.

Experiment 13.—An active pepsin was used, 100 mg. to the 10 c.c. A solution of an active commercial pancreatin was made containing in 200 c.c. 1,000 mg. of the ferment, 6 mg. hydrochloric acid and 200 mg. sodium chlorid. Fifty c.c. was incubated with 5 c.c. of the pepsin solution, boiled, through thirty minutes. It was then mixed with 50 c.c. of 1 per cent. starch and incubated until the iodin reaction disappeared. This required twenty-five minutes. A second 50 c.c. was mixed with 5 c.c. of active pepsin solution and incubated the same time, and then with starch. Forty minutes were now required for the disappearance of the iodin reaction.

These various tests show clearly some action of the pepsin on the amylopsin ferment. But with commercial specimens the behavior is not always so distinct, possibly because of the varying content of inorganic salts in the latter. As the limits of acidity within which amylopsin can act at all are only narrow, it may happen that the salts will neutralize the small amount of acid so that the pepsin in turn cannot be activated. This has been the case in some tests made, and here no definite effect in the direction of retardation could be affirmed. The subject, therefore, is one which is surrounded with a considerable number of difficulties. In the normal action in the body no action of the pepsin on amylopsin is likely because of the regularly occurring neutralization, but in view of the results of the experiments it is possible that some destruction of amylopsin might occur in the stomach. It is a question of reaction and excess of acid and the balance between the different phosphates as modifiers of acidity.

2. THE ACTION OF HYDROCHLORIC ACID AND PEPSIN ON TRYPSIN

We have here a question much like that discussed under 1. When the action of pepsin on trypsin is referred to it should be understood that pepsin in presence of hydrochloric acid is actually in mind in the great majority of cases. It has never been demonstrated that pepsin alone has any action on trypsin. The behavior of the acid alone has been frequently studied, but we have no very clear statement as to the behavior of pepsin and the acid under conditions which are important for the present inquiry.

Much of our knowledge of the digesting power of trypsin goes back to the time of Kühne, and many workers in the experimental field as well as on the commercial scale have followed his directions. Attempts to produce trypsin by the processes originally described by Kühne[14] yield at best a very weak product, with which it is difficult to make satisfactory experiments on the behavior of acids and alkalies. The effects are in all cases modified by the presence of relatively large amounts of accompanying inert proteins. Kühne spoke of his products as being able to digest bits of fibrin rapidly, but there is nowhere any evidence as to the relative weights of fibrin and ferment employed. It is likely that he must have used very much more of crude ferment than of fibrin, unless we accept the somewhat questionable hypothesis of Mays[15] that by accident, almost, Kühne secured extracts of the beef pancreas which were of a potence not since attainable by others. It was not thought necessary at the time of these earlier investigations to add anything to the pan-

14. Kühne: Verhandl. d. naturh. med. Ver. zu Heidelberg, 1877, p. 194; Untersuch. a. d. physiol. Inst. d. Univ., 1878, i.

15. Mays: Ztschr. f. physiol. Chem., 1903, xxxviii, 428.

creas powder or extract to bring about activation, and this fact has led to some question as to the real activity of the Kühne products.

Whatever their proteolytic power, however. Kühne mentioned that these ferment substances were extremely sensitive to acids. He stated that in the presence of 0.05 per cent. of hydrochloric acid their activity is still held, but above this destruction soon follows. This statement is sometimes misquoted and made to say that in 0.05 per cent. acid the ferment is destroyed. It was shown by other workers that a much greater acidity is necessary for actual destruction. Thus, Engesser asserted[16] that the pancreas powder made by him was able to resist an acidity of 0.5 per cent. hydrochloric acid. Of the same product Ewald says[17] that in presence of 0.3 per cent. hydrochloric acid it is able to dissolve fibrin to the condition of a turbid solution. These contradictory statements have long been quoted in the literature. It is likely that the last-named writers overlooked the fact that acid of the strength used is sufficient to bring about a partial solution of the fibrin in time. In hydrochloric acid of 0.3 per cent. strength fibrin swells to a jelly-like mass, and if enough liquid is present, slowly dissolves. It is also true that the pancreas powders as made by the Engesser process contained so much protein that they would probably neutralize a considerable amount of acid by combination.

It will be recognized that the question of the action of pepsin on trypsin must depend largely on the behavior of the acid itself with trypsin, since pepsin can be used only in acid medium. From his experiments Ewald concluded that trypsin is destroyed in acid solutions containing pepsin, and that the rapidity of destruction is proportional to the amount of pepsin present. Engesser admitted that any trypsin present as such would be quickly destroyed by acid and pepsin, but he held that in the pancreas powders the trypsin is present as a "zymogen," which has a greater resisting power toward acid and pepsin.

These views have mainly a historical interest at the present time, but it is proper to note that views almost as divergent on the two main contentions, the concentration of the acid which destroys trypsin, and the effect of pepsin and acid on trypsin, are still expressed in recent literature. Part of the difficulty in the problem seems to be due to the character of the various trypsin preparations employed by different investigators. The ferment activity of the pancreas powders made in the laboratory is often very low, and the same is true of most of the market products, as already intimated. It is commonly recognized that trypsin products, in general, have but a feeble action on raw egg albumin, but it

16. Engesser: Ztschr. f. klin. Med., 1880, ii, 192; Maly's Jahresber., 1880, x, 297.

17. Ewald. Ztschr. f. klin. Med., 1879, i, 615; Maly's Jahresber., 1880, x, 297.

is often overlooked that the action on boiled egg albumin is also relatively slow, although easily followed with moderately strong preparations. If tested by their action on coagulated egg, many market preparations would have to be pronounced practically inert, while with casein or fibrin a fairly sharp digesting power might be shown, as will be illustrated later. The behavior of acid toward trypsin may be best shown by the use of fibrin or casein as a test material, rather than egg.

In our efforts to determine the action of weak hydrochloric acid on trypsin we have used a number of products: first, a laboratory pancreas powder and a laboratory glycerol extract which had stood long enough to become active; second, samples of Gruebler's trypsin, and finally the products of some American manufacturing laboratories. The best of these, by far, is the "trypsin" of Fairchild Brothers and Foster, New York, which may be recommended for scientific investigations. Specimens which have come under our observation seem to have about four times the digesting power toward fibrin possessed by the Gruebler preparation, which is often taken as a standard for comparison. The "extract of pancreas" of the same firm, F. B. and F., is also very active, and in the experiments to follow will be often referred to.

As in the inquiry on the behavior of acid and pepsin toward amylopsin, we have also here the question of the action of acid alone to consider, and also other points as follows:

A. The action of water on trypsin.

B. The action of hydrochloric acid on trypsin.

C. The action of pepsin and acid on trypsin.

In studying the action of water or acid on trypsin we have determined the digesting power of the latter under different conditions, using egg albumin, fibrin and casein for the substratum in different experiments.

The egg albumin used for our experiments was prepared in this way: A very clear, light-colored, dry hen's egg albumin is brought into this country from China, and may be obtained from dealers. It seems to be used mainly by bakers, as are other similar products. Good samples of this product, which are not hard to secure, dissolve in distilled water to an opalescent liquid, free from lumpy particles and free from odor. When this solution is poured into a large volume of boiling distilled water, slightly acidified with acetic acid, a finely divided, perfectly white precipitate is secured, as with the solutions from fresh eggs, with this advantage, however, that the nitrogen content of the washed coagulum is pretty constant. With the very clean albumins which we have been able to secure for this purpose, the nitrogen of the washed coagulum amounts to something over 100 mg. for each gram of the original dry

egg. With one lot it was practically 106 mg. This weight is more nearly constant than may be obtained by using the coagulum from ordinary storage eggs, and serves well for digestion comparisons. We have found, however, that the results obtained from some other brands of dry egg albumin were not as satisfactory. Much of the American or German "egg" albumin cannot be used at all, as it is not freely soluble.

When fibrin is used, it is prepared by thorough washing in water and disintegration in a meat-chopper set to cut very fine. The chopped mass is soaked in glycerol until the larger part of the water is removed, which is hastened by pressing out thoroughly and treating with fresh glycerol. Finally, the mass is pressed as dry as possible and preserved at a low temperature in bottles containing a small amount of toluene. The product may be kept some weeks at a low temperature, and as it is comparatively dry and uniform it may be weighed directly for a series of digestions. For one of the last lots prepared in this way, and used for experiments to be quoted, the nitrogen content of 5 gm. was found to be 162.9 mg., in the mean, corresponding to about 20 per cent. protein. It has been noticed, however, that if left in a warm room autolytic digestion of the fibrin takes place rather readily, making the product unfit for use.

Casein was employed in the form of a sodium caseinate. We used a very pure casein made in the laboratory and dissolved it in weak sodium hydroxid solution, as will be explained below. The behavior of water will be studied first.

A. ACTION OF WATER ON TRYPSIN AT DIFFERENT TEMPERATURES

In pure aqueous solution trypsin becomes inactive much more quickly than pepsin, and markedly so at elevated temperatures. In 50 per cent. glycerol, trypsin may be kept through months. As to the exact effect of elevated temperatures there is a great lack of uniformity in the views of those who have reported on the subject. From our experience with a considerable number of trypsin preparations we have come to the conclusion that the salts present are probably the most important factors in modifying the stability.

According to the older experiments of Heidenhain,[18] trypsin begins to lose its activity when warmed in aqueous solution to 35 C., but this is denied by Kühne and others.[19] Biernacki[20] states that it becomes inactive in slightly alkaline solution at 45 C. Much of the discrepancy is doubtless due to the fact that different observers have worked with

18. Heidenhain: Maly's Jahresber., 1875, v, 176; Arch. f. d. ges. Physiol. (Pflüger's), 1875, x, 557.

19. Citation by Oppenheimer, Die Fermente, p. 114.

20. Biernacki: Maly's Jahresber, 1891, xxi, 248.

products of very different degrees of purity, but another fact of equally great moment is this, that the various trypsin preparations described in the literature seem to have been extremely weak products, their activities having been measured by the solution of small amounts of fibrin or coagulated egg in digestions carried through many hours.

In some experiments made to test this behavior in aqueous solution we have obtained the following results, after using the "extract of pancreas" and the "trypsin" of Fairchild Brothers and Foster, and the glycerol extract of our own production:

EXPERIMENT 14.—In Table 10 are given the digestion values obtained by incubating 100 mg. of the pancreatin (extract of pancreas) through thirty minutes at 40 C., 50 C. and 60 C., in 50 c.c. of pure water, after which treatment the liquids were brought to 40 C. and to each were added 100 mg. of sodium carbonate and 3 gm. of fibrin (nitrogen content 123.5 mg. in mean). The mixtures were incubated again through four hours at 40 C. Kjeldahl determinations were made on residue and filtrate.

TABLE 10.—DIGESTION VALUES OBTAINED WITH PANCREATIN PREVIOUSLY INCUBATED, FIRST WITH PURE WATER AND SECOND WITH SODIUM CARBONATE AND FIBRIN. (EXPERIMENT 14)

	Prelim. Digest.		Nitrogen, Milligrams		
Test No.	Temp. C.	Fibrin, Gm.	Soluble	Residue	Total
1	40	3	103.3	19.3	122.6
2	50	3	77.6	46.5	124.1
3	60	3	19.1	106.0	125.1

It will be seen that the effect of the preliminary digestions at the higher temperatures is to weaken greatly the activity of the pancreatin.

In a similar experiment with a preliminary incubation of thirty minutes at 40 C., 25 mg. of pepsin were added to the neutral solution, along with the 100 mg. of pancreatin. At the end of the half hour the mixture was acidified by the addition of 100 mg. of hydrochloric acid to the 50 c.c. Three gm. of the same fibrin were added and a new incubation was made at 40 C. At the end of forty-five minutes the digestion seemed to be complete. On filtering and making Kjeldahl determinations we found:

Soluble N	117.5 mg
Residue N	4.6 mg.
Total	122.1 mg.

Corrections were always made for the nitrogen of the ferments. It is evident that under the conditions the pancreatin has not destroyed or appreciably weakened the pepsin activity, as the fibrin digestion is here due to the latter ferment.

EXPERIMENT 15.—In a digestion test similar to 14 we used the trypsin, F. B. F., instead of the pancreatin, 50 mg. of trypsin being subjected to the preliminary incubation in 50 c.c. of water. The fibrin used was not from the same lot as the other. Final digestion, three hours. (Table 11.)

TABLE 11.—DIGESTION VALUES OBTAINED WITH TRYPSIN PREVIOUSLY INCUBATED, FIRST WITH PURE WATER AND SECOND WITH SODIUM CARBONATE AND FIBRIN. (EXPERIMENT 15)

Test No.	Prelim. Temp. C.	Fibrin Gm.	Nitrogen, Milligrams		
			Soluble	Residue	Total
1a	40	3	56.0	.....	.....
1b	40	3	57.9	47.0	104.9
2a	50	3	39.2	65.9	105.1
2b	50	3	38.9	66.1	105.0
3a	60	3	14.0	91.0	105.0
3b	60	3	14.2	91.0	105.2

It will be noted that in this experiment the time of final digestion is three hours in place of four, and that 50 mg. of trypsin were used in place of 100 of pancreatin. But it is evident that the trypsin has suffered relatively more than the other ferment at all temperatures. This seems to be true of the stronger ferments in general. As greater activity is secured, it is often at the expense of stability.

It has been said that glycerol solutions of trypsin are stable through long periods. On diluting such extracts with water we have found that they do not long preserve their activity. Even where sterilized water was used for the dilution, we found a marked decrease in action when the solutions stood over night at room temperature. The change may be in part due to the comparative absence of inorganic matters in such dilutions. At a temperature of 40 C. these pure aqueous solutions suffered greatly.

Pancreatin solutions (F. B. and F. as used above), however, when protected with toluene, were found to be fully active after standing forty-one hours at room temperature, as shown by fibrin digestion.

In another experiment the effect of longer heating was determined.

EXPERIMENT 16.—Fifty-mg. portions of trypsin were incubated at 40 C. in a volume of 50 c.c. of water through different periods, as shown below. Then to each flask 100 mg. of sodium carbonate were added and the incubation continued through three hours at the same temperature with the addition of 3 gm. of moist fibrin which had been kept in chloroform water. Kjeldahl tests were finally made to determine the amount of digestion. (Table 12.)

TABLE 12.—DIGESTION VALUES OBTAINED FROM TRYPSIN INCUBATED, FIRST WITH WATER AND SECOND WITH SODIUM CARBONATE AND FIBRIN. (EXPERIMENT 16)

Test No.	Prelim. Digest. Minutes	Nitrogen, Milligrams		
		Soluble	Residue	Total
1	30	48.4	49.0	97.4
2	45	50.4	....	....
3	60	53.5	42.6	96.1
4	90	42.6	....	....
5	120	47.5	50.4	97.9
6	180	42.8	54.9	97.7

It appears that at this temperature the effect of incubation is not much greater for a period of three hours than for half an hour. Some figures from a later experiment will suggest, however, that the best results are obtained by immediate digestion.

In conversation with manufacturers of pancreatins, we have learned that the effects of temperature and solution are recognized. It is often insisted that where tests are made on such preparations they should be carried out as quickly as possible after bringing the ferment into solution. In such investigations of comparative ferment value it is evident that a temperature of 40 C. is the most satisfactory and should not be exceeded.

The Effect of the Alkali

As tryptic digestions are always carried out in an alkaline medium, it is desirable to know the action of different amounts of alkali on the ferment. A few experiments only will be offered here, as we are concerned with the one alkaline substance only, sodium carbonate. It is usually stated that trypsin is not weakened or destroyed by moderate amounts of sodium carbonate, up to about 0.5 per cent. This depends, however, on the temperature, as the following tests show. While elevation of temperature weakens the trypsin greatly, almost complete destruction follows if much alkali is present. In the following experiments portions of 100 mg. of pancreatin in a volume of 50 c.c. were incubated with and without alkali through different times at temperatures of 40, 50 and 60 C. They were then brought to the same alkalinity and incubated with 3 grams of fibrin through periods, as shown, at 40 C.

EXPERIMENT 17.—Preliminary incubation with and without alkali, using pancreatin (F. B. and F.) and 3 gm. of pressed fibrin (N content 123.5 mg.)

TABLE 13.—DIGESTION VALUES OBTAINED WITH PANCREATIN PREVIOUSLY INCUBATED WITH AND WITHOUT ALKALI. (EXPERIMENT 17)

Test No.	Prelim. Alkali Mg.	Prelim. Inc. One Hr. at C.	Alkali Added Mg.	Final Inc. Hours	Result
1	...	40	100	6	Digestion completed
2	100	40	...	6	Digestion completed
3	...	40	100	6	Two-thirds digested
		50			
4	100	40	...	15	Slight digestion
		50			
		40			
5	...	50	100	15	No digestion
		60			
		40			
6	100	50	...	15	No digestion
		60			

In Nos. 1 and 2 (Table 13) the time and alkali effects are but slight, as the digestion is complete in six hours. In No. 3 the action of the higher temperature is shown in the preliminary digestion and when alkali is present at the outset the ferment seems to lose all its activity, practically, as shown in No. 4.

In Nos. 5 and 6 the temperature alone seems to be sufficient to stop digestion. These experiments were repeated a number of times with essentially the same results. Even when a smaller amount of fibrin was used and the stronger trypsin it was found that the prolonged heating with alkali above the temperature of 40 C. resulted in a marked weakening, or practical destruction of the ferment.

Finally, the effect of incubation with increasing amounts of carbonate was determined. The results show that the ferment is active in presence of a relatively large weight of this alkali.

EXPERIMENT 18.—Fifty-mg. portions of trypsin were incubated during thirty minutes with increasing amounts of carbonate, as shown. In three cases enough hydrochloric acid was then added to neutralize all but 100 mg. of the carbonate, leaving all of the same alkalinity as the first test. In the proper cases sodium chlorid was added to make all alike in this respect, and after the addition of fibrin the incubation was continued through two hours at 40 C. Kjeldahl tests were made on the filtrate and residue of the digestion.

In two of the numbers 5 and 6 (Table 14), there was no preliminary incubation with the carbonate. This was added with the fibrin at the beginning of the actual digestion. In the preliminary digestion the volume was 50 c.c., and was made to 80 c.c. in the final digestion in Nos. 1, 2, 3 and 4. In 5 and 6 the volume was 50 c.c.

TABLE 14.—DIGESTION VALUES OBTAINED WITH TRYPSIN PREVIOUSLY INCUBATED WITH VARIOUS AMOUNTS OF SODIUM CARBONATE. (EXPERIMENT 18)

Test No.	Mg. Prelim. Alk.	Mg. Final Alk.	Soluble Nitrogen, Milligrams	Residue Nitrogen, Milligrams	Total Nitrogen, Milligrams
1	100	100	77.1	22.7	99.8
2	200	100	79.4	22.1	101.6
3	300	100	84.6	16.0	100.6
4	500	100	85.7	16.0	101.7
5	...	100	98.0	4.5	102.5
6	...	500	98.0	4.2	102.2

The fact is brought out pretty clearly that in the preliminary incubation, the presence of the carbonate is protective of the ferment. It is also shown that where there is no preliminary incubation the final digestion is much nearer complete. Part of the apparent effect may, however, be due to the salt added, or formed in the other cases. The important fact which is shown is that the trypsin ferment exhibits the same activity in the presence of 1 per cent. of sodium carbonate that it exhibits with 0.2 per cent. In practice digestions may be carried out within these limits with like results, as long as the temperature is no higher than here.

B. THE ACTION OF ACID ON TRYPSIN

As our object was mainly a practical one, to determine the possible part played by pepsin in aiding the acid destruction of trypsin, we have confined ourselves to a study of the behavior of hydrochloric acid. To learn the effect of pepsin on trypsin it is, of course, necessary to fix first the action of the acid with which the pepsin works and for this purpose hydrochloric acid alone comes practically into consideration. Trials have been made with both egg albumin and fibrin, and we shall quote

first a few of the egg-digestion experiments to show an important point regarding the behavior of this form of protein. The sodium carbonate mentioned was always taken as anhydrous. In all cases a correction was made for the nitrogen of the ferment itself.

EXPERIMENT 19.—Fifty mg. of F. B. and F. trypsin plus coagulum from 1 gm. of egg (106 mg. nitrogen) plus 50 mg. of sodium carbonate in volume of 50 c.c. Digested ten hours at 40 C. The undissolved egg was filtered off.

Soluble N = 25.5 mg.

EXPERIMENT 20.—Fifty mg. of same trypsin plus 30 mg. of hydrochloric acid made to 50 c.c. with water and incubated one hour at 40 C. Then 100 mg. of sodium carbonate was added with the coagulum from 1 gm. of egg and the digestion continued through ten hours at the same temperature.

Soluble N = 27.2 mg.

EXPERIMENT 21.—Fifty mg. of same trypsin, plus coagulum from 1 gm. of egg, plus 200 mg. of sodium carbonate made to volume of 50 c.c. and digested ten hours at 40 C.

Soluble N = 14.8 mg.

It is apparent from these tests that egg albumin is but slowly digested by trypsin. This fact has been occasionally referred to in the literature, but it is not clearly recognized. Some observers have actually recommended the use of uncoagulated egg white for such digestion tests to determine the value of trypsin, but it is absolutely unsuitable for the purpose, as will be pointed out below. The fibrin digestion is much more rapid, as a few experiments will indicate.

EXPERIMENT 22.—Fifty mg. of same trypsin, plus 100 mg. of sodium carbonate, plus 3 gm. of fibrin (97.7 mg. of nitrogen) made to 50 c.c., and digested at 40 C. The fibrin was all dissolved in thirty minutes.

EXPERIMENT 23.—Fifty mg. of Gruebler's trypsin, plus 100 mg. of sodium carbonate, plus 3 gm. of the same fibrin, made to 50 c.c. volume and digested at 40 C. Over two hours required for complete solution.

The last experiments show the marked difference between the two kinds of trypsin, which results were duplicated by many other observations, but they show also the ease of fibrin digestion as compared with that of egg. In one of the egg experiments a small amount of hydrochloric acid was employed in a preliminary digestion with the trypsin. This amount of acid did not appear to have any inhibiting effect as far as the subsequent digestion, after neutralization, was concerned, using either egg or fibrin. With such strengths of acid in preliminary digestion the solution of the egg was always but partial, as was the case when no acid was used. But the fibrin digestion could easily be brought to completion after such treatment. It was found that a still greater degree of acidity in the preliminary incubation seemed to have but a slight action on the trypsin, when tested with fibrin, and this was shown quantitatively with the F. B. and F. ferment in the following tests:

EXPERIMENT 24.—A series of solutions was made containing in 50 c.c. 50 mg. of the trypsin and increasing weights of the acid up to 100 mg. These solutions were incubated thirty minutes at 40 C. and then mixed with enough sodium carbonate to neutralize the acid and leave 100 mg. of the carbonate present in each case. Enough sodium chlorid was added to four of the mixtures to make the content the same as in the mixture with the greatest acid originally. Finally 5 gm. of fibrin, containing 162.8 mg. of nitrogen, was added to each mixture and all were incubated five hours at 40 C. The rate of digestion appeared to be nearly uniform. At the end of the period all of the solutions were filtered and Kjeldahl determinations made on filtrate and residue. The results were as shown in Table 15.

TABLE 15.—DIGESTION VALUES OBTAINED WITH TRYPSIN PREVIOUSLY INCUBATED WITH ACID, THEN MIXED WITH SODIUM CARBONATE. (EXPERIMENT 24)

			Nitrogen, Milligrams		
Test No.	HCl Mg.	Trypsin Mg.	Soluble	Residue	Total
1	60	50	156.6	7.8	164.4
2	70	50	152.3	11.5	163.8
3	80	50	151.0	11.9	162.9
4	90	50	151.4	13.4	164.8
5	100	50	151.4	10.6	162.0

It will be seen that a preliminary digestion in a medium containing 0.2 per cent. of hydrochloric acid does not destroy or practically retard the activity of the trypsin. The digestion, after neutralization, is as complete in the mixtures which contained the largest amount of acid as it is in the one with the smallest amount. The weights of total nitrogen recovered are not quite uniform. This is doubtless due to the fact that absolute uniformity in the nitrogen content of the moist fibrin could not be secured.

EXPERIMENT 25.—The preceding experiment was repeated, using a new and much dryer preparation of fibrin. Three-gram samples contained, in the mean, 168.8 mg. of nitrogen. In these tests the acid added in the preliminary incubation was greater, as shown in Table 16.

TABLE 16.—DIGESTION VALUES OBTAINED AS IN TABLE 15, EXCEPT FOR USE OF A DRYER FIBRIN AND LARGER AMOUNT OF ACID. (EXPERIMENT 25)

			Nitrogen, Milligrams		
Test No.	HCl Mg.	Trypsin Mg.	Soluble	Residue	Total
1	100*	50	18.2	154.0	172.2
2	120	50	129.3	37.5	166.8
3	140	50	139.4	25.8	165.2
4	160	50	130.7	43.5	174.2

* + 25 pepsin.

In the first of these tests the preliminary incubation of thirty minutes with pepsin, in addition to the acid present, resulted in a marked lowering of the efficiency of the trypsin in the final fibrin digestion. In the other cases the acid digestion alone, with amounts of acid reaching 0.32 per cent., shows a slight impairment only of the activity of the trypsin. For our purpose it was not thought necessary to carry the acidity beyond this last value, or to increase the time of preliminary

incubation beyond thirty minutes. We have kept in mind the practical bearing of these questions on problems connected with actual conditions in the human body.

It is evident from the preceding tests that trypsin, even when not protected by a great excess of protein, is able to withstand an acidity of over 0.3 per cent. of hydrochloric acid through thirty minutes at 40 C. Somewhat similar results have recently been reported by others, to be referred to, but the weights of protein used in the final tests of the residual trypsin activity have usually been so small as to leave the question really very much in doubt. It will be recognized that in these experiments we are not dealing with the matter of the digestion by means of trypsin in acid solution. It has been shown by many observers that in presence of acid the activity of trypsin is practically nil.[21] We are simply concerned with the effect of acid on trypsin in absence of much protein.

In this connection it is in order to refer to some similar experiments carried out with casein as the substratum. Casein may be made in very pure dry form and serves as an excellent standard substance. We have used it in different ways and in this series of tests by the method of Fuld,[22] according to which a weak sodium caseinate is prepared and treated with the ferment until it no longer gives a precipitate with dilute acetic acid. Ordinarily the method has been worked with very small weights of casein, usually 4 mg. We found a larger weight preferable as a substratum, as it permits the use of stronger ferment solutions. One set of experiments may be quoted as illustrating the whole series of tests.

EXPERIMENT 26.—Our casein solution was made with the minimum amount of alkali required for the formation of the acid salt.[23] As a trypsin we used in this series the "Extract of Pancreas" of Fairchild Brothers and Foster, already referred to. Six test-tubes were charged with amounts of the pancreatin, as shown below, and to each was added 5 c.c. of 0.2 per cent. hydrochloric acid. All the tubes were incubated one-half hour at 40 C., then neutralized exactly. To each tube 6 c.c. of 0.2 per cent. casein solution was added and these, in turn, were incubated one hour and then the tests for unaltered casein were made by adding to each tube three drops of the acetic acid reagent (1 part of acid, 50 parts of alcohol, 49 parts of water). Table 17 shows the relations. It is seen that even with the weakest pancreatin concentration, and after the half-hour incubation with acid, the casein is completely changed in the final digestion.

21. See, for example, Chittenden and Cummins: Am. Chem. Jour., 1885, vii, 49.

22. Verhandl. d. Ver. inn. Med., 1907, Maly's Jahresber., 1907, xxxvii, 446; 1908, xxxviii, 375.

23. Long: Some Investigations on Salts of Casein, Jour. Am. Chem. Soc., 1906, xxviii, 372.

TABLE 17.—DIGESTION VALUES OBTAINED WITH TRYPSIN TESTED AGAINST CASEIN. (EXPERIMENT 26)

Test No.	Pancreas Ext., Mg.	Casein, Mg.	Result
1	2.0	12	Complete digestion
2	1.3	12	Complete digestion
3	0.8	12	Complete digestion
4	0.5	12	Complete digestion
5	0.3	12	Complete digestion
6	0.0	12	No apparent change

The results of this method fully confirm those previously obtained by the fibrin digestion.

C. THE ACTION OF ACID AND PEPSIN ON TRYPSIN

Having discussed the action of weak hydrochloric acid on trypsin, we now come to the practical question of the behavior of pepsin and acid working together. As shown above, we are concerned with hydrochloric acid only, as acids of lower ionization do not come into the question at all. In fact, it is essentially with the hydrogen concentration from this acid that we have to deal, and our experiments have been so arranged as to secure the concentration indicated by the percentage weights used. To this end we have used with the acid only small weights of strong ferments and no protecting protein to combine with the acid. With the addition of the pepsin in the preliminary incubation the same plan will be followed.

At the outset it was intimated that the few investigations which have been carried out to shed light on this subject have not furnished very concordant or satisfactory results. Some of them have been roughly qualitative only, not quantitative. Much of the trouble has been with the weak trypsin products employed in the tests, making it necessary to incubate through relatively long periods, and thus introduce the disturbing time and temperature factor. The weak ferments, with high protein content, combine themselves with so much acid that a further disturbing factor is introduced. It is practically useless to employ the ordinary "pancreatins" of the market in such investigations, as their trypsin content is always low, and often nil.

Among the more recent investigations in this direction which may be quoted, two will serve as illustrations.

Some extended observations have been published by Wroblewski, Bednarski and Wojczynski[24] on the behavior of ferments toward each other and toward acids and alkalies. Some of their tests were carried out with raw egg white as substratum and others suffer from other errors of method or manipulation, so that many of the results are quite meaningless. In some of their experiments the weights of ferment and egg

24. Wroblewski, Bednarski and Wojczynski: Beitr. z. chem. Phys. u. Path., 1902, 1, 288.

were as two to one, apparently. From one of their series of experiments the conclusion is drawn that hydrochloric acid has a destructive action on trypsin and that pepsin increases the extent of this action. The experiments were carried out in this manner: Ten c.c., usually, of a 2 per cent. trypsin solution was acidified with hydrochloric acid to concentrations of 0.056 to 1.12 per cent. in different tests, and treated with pepsin, usually 0.1078 gm. The mixtures were incubated at 37 C. through six hours, neutralized, diluted to the same volume, brought to an alkalinity of 0.1 per cent. soda and mixed with 3 c.c. of "1 to 5" egg albumin solution for final digestion. How long the digestion was continued is not clearly stated. The weight of undigested albumin after the treatment is given for a number of experiments. This was obtained by filtering off the coagulum produced by boiling the liquids after digestion with the "right amount" of 10 per cent. acetic acid, drying and weighing. But the numerical values given are so irregular as to fail utterly to justify the conclusion of the authors in this case, as well as in others handled in this long paper.

Another paper, more recent than the work first cited, was read at the Indianapolis meeting of the American Chemical Society in June, 1911, by Zimmerman.[25] This paper handles the question of the mutual action of certain ferments, among them pepsin and pancreatin, and the conclusion is reached that the action of pepsin on the tryptic ferment is not marked. The reasoning of the author is not always easy to follow, and what is given as to the method does not always explain the results reported. As this paper has attracted some attention it is necessary to go over certain details, as given. In some of the experiments reported it appears that pepsin and pancreatin were mixed in 50 per cent. glycerol and allowed to stand through rather long periods. When tests were finally made it was found that both ferments were active; that is, neither had destroyed the other. No details of quantitative tests are given, however, and it is impossible to say how far one or the other may have been weakened. It is also to be remembered that reactions in glycerol are in general much weaker than in water solution, because of diminished ionization, and for this reason the comparison is not practically a fair one. Many liquids besides glycerol would have the same effect. No conclusion can be drawn from such experiments as to the behavior in the stomach, the practical question in most cases.

Zimmerman finds further that in 50 per cent. glycerol solution trypsin retains its activity in presence of 0.35 per cent. hydrochloric acid. This is probably true. But attention is not drawn to the fact that relatively large weights of the pancreatin powder are mixed with the acid, in some

25. Zimmerman: Jour. Indust. and Engin. Chem., 1911, iii, 750.

cases 2 gm. or more being mixed with 25 c.c. of the acid. It is evident that the action of the acid must be materially modified by the relatively great weight of inert protein present with the ferment. This is true of most of the mixtures which Zimmerman describes. The weight of ferment taken is out of all proportion to the acid or alkali the effect of which is to be determined. This is especially true of the mixtures "containing 10 per cent. each pepsin and pancreatin" and containing varying amounts of acid. For 25 c.c. we have here, practically, 5 gm. of protein to hold the acid. It is not to be wondered at that under these conditions and in presence of glycerol the ferments should have but little action on each other. While the author's conclusions are doubtless correct as far as the mixtures observed are concerned, they do not appear to be practically applicable.

Our own experiments bearing on the question follow. It was shown in one of the series of tests given above on the action of hydrochloric acid on trypsin, Experiment 25, that when pepsin was present with the acid in the preliminary incubation a marked weakening of the trypsin occurred, as shown by the action of the neutralized mixture on subsequently added fibrin. This experience was many times duplicated, as illustrated by this experiment.

EXPERIMENT 27.—A solution was made containing in 50 c.c. 100 mg. of hydrochloric acid, 50 mg. of F. B. F. trypsin and 25 mg. of active pepsin (fully 3,000 test). This solution was incubated thirty minutes at 40 C. At the end of the time it was treated with enough sodium carbonate to leave an excess of 100 mg. after neutralization. This neutralization left nearly 162 mg. of sodium chlorid. Then 5 gm. of moist fibrin (with nitrogen content of 162.8 mg. in the mean) was added and digested five hours at 40 C. The soluble nitrogen obtained was 85.3 mg. and the residue left contained 77.8 mg. In similar experiments without the pepsin the soluble nitrogen from the same amount of fibrin was over 150 mg.

Experiments like the preceding show a distinct action of pepsin on trypsin. The acid present is relatively high in comparison with the total protein, so that most of it must remain actually free, and not fully combined, as in some of the experiments reported by Zimmerman.

In this connection another set of experiments in which the final test was made by the Fuld casein method may be reported, with the following as the illustration:

EXPERIMENT 28.—Twenty mg. of F. B. F. pancreatin and 5 mg. of active pepsin were incubated in 5 c.c. of 0.2 per cent. hydrochloric acid through thirty minutes. Then the acid was neutralized and portions corresponding to 2, 1.3, 0.8, 0.5 and 0.3 mg. of the pancreatin were measured out and incubated one hour with 12 mg. of casein (as sodium caseinate) in a total volume of 10 c.c. When the acetic acid test was made much of the casein was found in all the incubated flasks, showing that the pepsin had weakened or destroyed the pancreatin.

These experiments were followed by a number of others carried out in the same manner, but with longer digesting periods. In four-hour

periods the digestion of the casein was found to be practically complete, suggesting a weakening, or partial destruction, rather than a complete destruction of the trypsin by the pepsin.

Following these tests a considerable number of others were made in a different way. In some of these the coagulum from the solution of dried egg albumin was used and in this manner.

EXPERIMENT 29.—Fifty mg. of trypsin, 30 mg. of hydrochloric acid, 50 mg. of pepsin in boiled solution and the coagulum from 1 gm. of egg were made to 50 c.c. and incubated one hour at 40 C. The mixture was then neutralized with 100 mg. of sodium carbonate and incubated through ten hours.

Soluble N, corrected for N of ferments = 36.4 mg.

EXPERIMENT 30.—Same as last with this exception: the pepsin solution added was not boiled.

Corrected soluble N = 26.9 mg.

The acid content is here so low that little or no peptic digestion of the egg might be expected. The digestion is probably wholly due to the trypsin, which seems to be weakened to some extent by the pepsin in the second experiment.

EXPERIMENT 31.—In a series of flasks 50 mg. of trypsin, 25 mg. of pepsin (both F. B. F.) and increasing amounts of hydrochloric acid were made to a volume of 50 c.c. The flasks were incubated at a temperature of 40 C. through thirty minutes and neutralized with enough sodium carbonate to leave an alkalinity of 0.2 per cent. of the carbonate. Salt was added to four of the flasks to make the content the same as that in the one with the highest original amount of acid. To each one 5 gm. of fibrin was added (mean nitrogen content 160.1 mg.) and a further incubation of six hours was made. The amounts of soluble nitrogen were determined as in Table 18.

TABLE 18.—DIGESTION VALUES OF TRYPSIN PREVIOUSLY INCUBATED WITH PEPSIN AND HYDROCHLORIC ACID, THEN NEUTRALIZED. (EXPERIMENT 31)

Test No.	HCl, Mg.	Soluble N., Mg.
1	25	9.25
2	30	17.57
3	35	7.77
4	40	3.33
5	50	6.79

Controls without the pepsin showed complete digestion of the fibrin.

There appears to be here a marked destruction of the trypsin. In another set a new fibrin was used with mean nitrogen content for 5 gm. = 167.4 mg. This was incubated with the pepsin and trypsin in some cases, and in others was added after the preliminary incubation, as shown below.

EXPERIMENT 32.—In a number of flasks different weights of trypsin and pepsin (as above) and acid were made up to 50 c.c. and incubated thirty minutes at 40 C. In 1 to 6 (Table 19) the fibrin, 5 gm., was added before the incubation, and in 7, 8 and 9 after the incubation and neutralization. In all cases enough sodium carbonate was added to make the final alkalinity equal 0.2 per cent. The period of incubation after adding the alkali was four and one-half hours.

TABLE 19.—DIGESTION VALUES OF TRYPSIN INCUBATED AND NEUTRALIZED BEFORE AND SOME AFTER ADDITION OF FIBRIN

No. Test	Trypsin Mg.	Pepsin Mg.	HCl Mg.	Fibrin Gm.	Nitrogen, Milligrams Soluble	Residue
1	100	25*	35	5	159.6	7.6
2	100	...	35	5	157.9	8.1
3	100	25	35	5	159.9	6.3
4	100*	25	35	5	88.4	74.8
5	...	25	35	5	105.7	60.3
6	...	...	..	5	.....	167.4
7	50	...	40	5	lost	5.2
8	50	...	50	5	157.8	10.3
9	100	25	35	5	42.9	122.6

* The ferment was added in a boiled solution.

Nos. 1 and 2 of Table 19 show the digestive action of trypsin alone, as in one case no pepsin is present and in the other killed pepsin. We have a nearly complete solution of the fibrin. No. 3 shows practically the same result, and apparently no destruction of trypsin because this is protected by the fibrin present. There is also some digestion by the pepsin in acid solution, which obscures the effect of the trypsin to some extent. This is a mixed digestion. In No. 4 we have the effect of the pepsin and acid alone, as the accompanying trypsin was boiled. No. 5 shows a somewhat similar condition, indicating that the pepsin alone can digest but a part, less than two-thirds of the fibrin. No. 6 shows the nitrogen content of the fibrin. In Nos. 7 and 8 we have this difference in condition, that the fibrin was added after the preliminary acid diggestion, with no pepsin present. The trypsin remained active enough to digest the fibrin finally. In No. 9 trypsin and pepsin are digested together without protecting fibrin. When the latter is added later the trypsin seems to be now too weak to digest much of it. This coincides with some of the earlier tests in indicating, under these conditions, a partial destruction of trypsin by pepsin.

A much more complete destruction of trypsin is shown if the digestion in presence of acid is continued beyond an hour. As long as the temperature of digestion does not go above about 40 C., the effect of incubation in water alone is not noticeable for several hours, with most trypsins, but there seems to be some difference in products from different sources in this respect, due possibly to salt content. In weak acid, up to 0.2 per cent. hydrochloric acid, the destruction of the trypsin is not rapid.

A large number of experiments have been made to show the effect of acid and pepsin when the preliminary incubation is prolonged. In these tests the coagulum from 1 gm. of dry egg, prepared as explained above, was employed to measure the action. The mean nitrogen content of a gram was 106 mg. A few of the results from such experiments are sufficient to quote, as those from the use of larger amounts of acid or from longer incubation show, of course, the same destructive action.

EXPERIMENT 33.—In a number of flasks hydrochloric acid, pepsin and trypsin, in amounts shown below, were made up to a volume of 50 c.c. and incubated through two hours at 40 C. In some of the tests the trypsin was added in boiled solution. Then sodium carbonate was added to neutralize the acid and leave

0.2 per cent., more trypsin added, boiled or unboiled, and the coagulum from 1 gm. of egg. A new incubation was carried out through four hours, when the amount of soluble nitrogen was determined. A correction was made for the nitrogen of the ferments added. This is assumed to be all soluble.

TABLE 20.—DIGESTION VALUES OF TRYPSIN AFTER PROLONGED INCUBATION WITH PEPSIN AND ACID. (EXPERIMENT 33)

No. Test	Pepsin Mg.	Trypsin Mg.	HCl Mg.	Trypsin After Incubation Mg.	Nitrogen, Soluble,	Milligrams Residue
1a	25	100	20	100*	.....	108.4
1b	25	100	20	100*	.....	106.7
1c	25	100*	20	100	95.4	16.2
1d	25	100*	20	100	90.4	18.5
2a	25	200	20	200*	.....	108.6
2b	25	200	20	200*	.....	108.9
2c	25	200*	20	200	62.0	44.1
2d	25	200*	20	200	†	†
3a	25	100	50	100*	.....	103.9
3b	25	100	50	100*	.....	109.5
3c	25	100*	50	100	71.1	28.3
3d	25	100*	50	100	68.9	30.1

* Trypsin in boiled solution.
† Lost.

The trypsin used in the experiments Nos. 2a to 2d was a much weaker product than that used in the other cases.

These experiments show that the trypsin freshly added digests the egg very well, but after contact with acid and pepsin through two hours it is inert. We have found that some samples of trypsin or pancreatin heated with the acid of the strengths here used become inert, even when no pepsin is used. But the process of manufacture leaves some of these products with a larger amount of inorganic salts present. Three well-known brands of pancreatin of American manufacture show 6.7, 7.6 and 14.8 per cent. of ash, respectively. These facts must be kept in mind when comparisons of such products are made.

It is evident from the sum of the experiments quoted that, *in vitro*, the destructive action of acid on trypsin is much accelerated by the presence of pepsin. This action is modified to some extent by excess of protein present, and it is this fact which makes a definite statement as to what happens in the body somewhat difficult. An accumulation of acid beyond that necessary to combine with protein to form acid albumin would undoubtedly interfere with the activity of trypsin present at the same time. There appears to be no recovery of trypsin after neutralization of the inhibiting acid. In the actual conditions in the stomach the acid ordinarily accumulates beyond the protein-saturating point and would then be able to work on trypsin ingested. These experiments appear to uphold the view held by many physiologists that under practical conditions pepsin is antagonistic to the tryptic ferment.

In the course of our studies of these ferments we have had occasion to examine most of the pancreatins of American manufacture. There are numerous brands on the market, but many of them come from the same source. It has been found that while some of these have a fair amylolytic action, their tryptic power is usually low. Some are, indeed, quite inert. This must follow from the careless process of manufacture often followed, which may consist in the rapid drying of the pancreas gland, the removal of most of the fat by extraction with some convenient solvent and final grinding to powder. The temperature of drying undoubtedly is high enough to destroy the sensitive ferments. The low wholesale price at which some of these powders are sold would point to a very hasty process of manufacture. The tests prescribed by the Pharmacopeia for pancreatin do not encourage a higher strength than that usually found. The standard is low enough to suit the indifferent manufacturer. In this respect there is a curious contrast between the treatment of pepsin and pancreatin which certainly calls for correction.

These criticisms hold for the edition of the Pharmacopeia now in use, and they seem to hold for the revision of the work soon to appear. The conversion of 25 parts of starch, made into paste, to the colorless endpoint by 1 part of ferment, in five minutes, is a requirement too low to have much practical value. It is not a fair standard, since there are a number of commercial pancreas products known which have a far greater degree of activity. The Pharmacopeia describes pancreatin as containing the other ferments of the pancreas, as well as the amylopsin. Why is no proteolytic standard adopted? The market grades will remain weak as long as the Pharmacopeia is silent on this point. Possibly all concerned recognize the seeming futility in making a product the strength of which is almost certain to be impaired, and perhaps destroyed, in passing through the stomach.

This point should be kept clearly in mind by physicians that the pancreatins of the market are mostly weak, and further, that if they were strong there is no certainty that they would be of much more use in actual practice, as commonly administered. The question may well be asked: Except for the purpose of curdling and partly predigesting milk, why should pancreatins be used at all? As digestive agents their usefulness is certainly of a very low order, and it is likely that there would be no practical loss in dropping pancreatin entirely from the Pharmacopeia.

As illustrating the actual strength of the best known brands in this country, the following table is given. The samples tested were collected fresh from the manufacturers in January, 1913, and examined at once as to their behavior with starch and fibrin. A year later, January, 1914, they were tested again. It has been repeatedly pointed out that the

presence of certain inorganic substances is essential for the development of the maximum activity of the amylopsin. In many cases the process of manufacture leaves enough of this in the finished product, usually chlorid and phosphate, but occasionally this is not the fact and more salt should be added by the analyst. The Pharmacopeia fails in this very important provision, and the failure may sometimes lead to an improper rating of a product examined.

The starch-digesting power of the pancreatins will be given first, the different products being designated by number rather than by name. The numerical values given show the number of minutes required to convert 500 parts of starch to the colorless end-point by 20 parts of the ferment; that is, the low standard of the Pharmacopeia is taken for comparison. The amount of salt added was sufficient to insure a concentration of 0.1 per cent.

TABLE 21.—STARCH-DIGESTING POWER OF PANCREATIN

	Tests Made in 1913		Tests Made in 1914	
Number	No Salt Added	Salt Added	No Salt Added	Salt Added
1	3	3	...	3
2	15	5	105	27
3	5	4	15	10
4	60	15	...	10
5	5	4	...	14
6	4	4	...	5
7	10	4	*	80
8	10	4	...	25
9	5	4	...	7
10	5	4	...	4
11	†	..	..	..
12	10	4	...	..
13	15	4	...	..

* Inert.

† Low in amylopsin.

It will be seen that, when perfectly fresh, all of these samples, except one, satisfy the requirement of the Pharmacopeia, as long as sufficient salt is present. In absence of added salt half of the samples are found to be weak by this very lenient test. One year later over half of the samples are found to be weak, even with the salt, and some had become practically inert. Three samples retained their former value, and these were characterized by a high original content of inorganic matters. This is evidently a factor in the keeping quality as well as in the residual activity. It may be recalled that there are pancreas products on the market which are not sold under the name of pancreatin and which have four times the starch-converting power here shown. It

is therefore evident that in actual digesting value and in keeping qualities some of these products are far from satisfactory.

We find a somewhat similar situation when we come to the consideration of the proteolytic power, as measured by the action on moist fibrin. In each test, in the first examination, 25 mg. of ferment, 2 gm. of fibrin and 50 c.c. of 0.2 per cent. sodium carbonate solution were incubated at 40 C. until the digestion was practically complete, as shown by the disappearance of all but stray flakes of the fibrin. In the tests made in 1914, 100 mg. of the ferment were taken in each case because the products had become weaker.

TABLE 22.—Proteolytic Power of Pancreatins

	1913 Tests	1914 Tests			
No.	Digestion Time	Change in One Hour	Change in Three Hours	Primary Albumose	Secondary Albumose
1	80 minutes	Very slight	Slight	Slight	Trace
2	Apparently no change	Very slight	Very slight	None	None
3	120 minutes	Very slight	Very slight	Trace	Trace
4	720 minutes	Very slight	Very slight	Trace	None
5	100 minutes	Very slight	Very slight	Trace	None
6	40 minutes	Slight	Marked	Some	None
7	100 minutes	Slight	Marked	Some	Trace
8	Very little change	Very slight	Very slight	Trace	None
9	40 minutes	Slight	Slight	Trace	Trace
10	12 minutes	Nearly all	All gone	Large	Large
11	8 minutes	All gone	All gone	Large	Large
12	70 minutes				
13	105 minutes				

The terms primary and secondary albumose are here used in the old sense, the first denoting the precipitation by half saturation with ammonium sulphate, while the notation under secondary albumose indicates the result by completely saturating the filtrate from the primary albumose with the sulphate. The results are mostly trifling in amount.

In examples 10 and 11, Table 22, we see what may be expected from a really active tryptic preparation, but in most of the other cases the amounts of fibrin digested by the year-old specimens are very low, and probably so low as to merit no consideration. Even when fresh, most of the pancreatins are slow in their tryptic action and two are inert. The manufacturers of pancreatin have not yet learned the secret of making a product with a reasonable degree of tryptic activity, if this is indeed considered worth while in view of the probable partial destruction in the stomach.

It is evident that samples of pancreatin which have stood on drug-store shelves a year or more are liable to be very low in tryptic as well as in starch-digesting power. The question naturally presents itself, What, then, is the practical value of these preparations? Certainly, pancreatin, as now made, leaves much to be desired. It has yet to earn a place in the category of remedies which are actually useful.

Northwestern University Medical School.